

Winter School

on

"Winter School on Climate smart agricultural technologies for resource conservation and increasing farmer's income"

(19 Nov – 9th Dec, 2019)

Sponsored by

Indian Council of Agricultural Research, New Delhi

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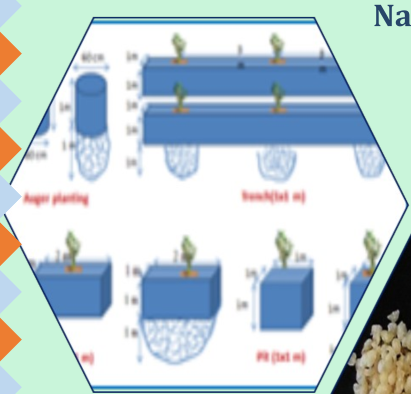
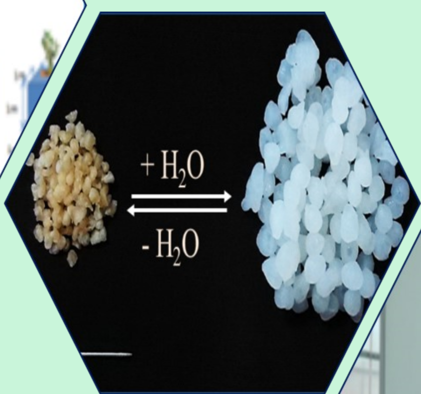

Manual

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National Institute of Abiotic stress Management (NIASM)
Malegoan (Khurd), Baramati-413115 Pune
(Maharashtra) India

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NIASM

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Training Manual

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Content

Chapter- 1

Plant bio–regulators for improving crop productivity and post–harvest quality under climate change

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1.1. Abstract

Reliable large−scale agriculture production is an essential component of global food security; however, sustained efforts are needed to ensure optimized resilience under diverse crop stress conditions. Climate changes are expected to increase the frequency and intensity of both abiotic and biotic stress. Plant bio−regulators (PBRs) play an important role in stress resilience, yet the concentration and composition of these PBRs are also dependent on climate variables. In this research, impact of PBRs on crop yields, water productivity and post−harvest quality of major crops (wheat, soybean, sorghum, onion and eggplant) under water scarce conditions was studied. Potential PBRs included: potassium nitrate (KNO_3), salicylic acid, sodium benzoate (SB), thio−urea (TU), gibberellin (GA_3) and ortho-silicic acid (OSA) were applied exogenously at 3−4 critical growth stages of specific crop as main treatments. The uniquely designed line−source sprinkler system (LSS) was used to apply varied levels of irrigation water (IW) ranged between 0−1.0 times the CPE (cumulative open pan evaporation) as sub–treatments. The application of PBRs mitigated water stress and significantly improved yields, water productivity and post−harvest quality. The PBRs maintained higher leaf water content, lower canopy temperature, modulated the stomatal opening and ultimately the source–sink relations thereby improving the yield and productivity under deficit irrigation. Particularly PBRs like thio–urea (10 mM), sodium benzoate (100 mg L^{-1}), KNO_3 (1.5%) and salicylic acid (10 μM) were found effective to mitigate water stress in wheat, sorghum, onion and eggplant, respectively. Thus relative response of PBRs is highly specific environment conditions and varies with crop to crop. PBRs also helped to improve significantly physicochemical and functional quality characteristics *viz.*, rehydration ratio, protein content, total soluble sugar, total phenolics content and pyruvic acid in water deficits. It is concluded that conjunctive use of PBRs along with supplemental irrigation present viable water stress mitigation strategy, improved crop quality and water productivity under the climate change.

1.2. Introduction

The crop productivity is exposed to different types of abiotic stresses (heat, cold, drought, flood, salinity, mineral deficiency, toxicity, chilling or freezing stress etc.) and potential yield are seldom achieved with stress. The present challenges *viz.*, water and soil pollution, urbanization, global climate change etc. further add up to the situation. Overall effect of abiotic stresses depends on the intensity and length of stress varies with growth stages and cultivars. Abiotic stresses are linked with natural phenomenon and their scale varies at temporal and special dimension. The changing climate poses serious threats to global agricultural production and place unprecedented pressures on the sustainability of agriculture industry. On the other hand, population growth and health–conscious consumers demand more and better food products. In addition to restricting carbon emission and conserving resources, adaption of abiotic stress mitigation strategies for sustainable vegetable production will be the single most important step that we take in the future.

Water stress and moisture availability in soil exert great influence on plant growth through direct and indirect effects viz., root development, vegetative growth, uptake and mobilization of nutrients. It also affects turgidity, normal metabolism, cell division and enlargement which influence overall crop growth response. Bio−regulators (PBRs) play an important role to control the physiological metabolic activities in crops under water stress conditions. The research needs to be focused on increasing water use efficiency and control of metabolic activities in crops through use of PBRs under varied irrigation water regimes in different crops cultivated in water stressed regions. For this purpose, creation of large number of water levels which vary systematically from one end of single plot to the other are needed. The line source sprinkler technique facilitates the application of progressively decreasing amounts of water at increasing perpendicular distance from the line source (Hank, 1980). Therefore present research aimed to develop of crop water functions and impact of bio−regulators and supplemental irrigation on productivity and post-harvest qualities of different crops grown under different water stressed regions using line source sprinkler plot irrigation system as stress mitigation strategy for climate resilient agriculture.

Crop yield is primarily water-limited in arid and semi-arid regions. Under limited irrigation water, reduction in grain yield and its post-harvest quality due to restricted water availability depends on degree, duration and timing of imposed water deficit. Many studies on plant responses to water deficits (stress) were carried out by investigators concerned with agricultural production, environment and resources, and macroscopic physics of soil, plant, and atmospheric water. As expected, the physiological and metabolic aspects of these studies were often weak and, on the other hand, studies carried out by metabolism-oriented biologist's frequently slighted important physical facets. Nevertheless, laudable investigations, especially during the last few years, have been sufficient to warrant optimism about substantial progress in the near future. Overall research on crop resistance or tolerance to abiotic stresses has not received much attention. Therefore, well understanding of plant responses to the interactive effect of water and nutrients deficits, how these deficits may theoretically affect plant processes using PBRs are the needs of the future line of research.

The helpful role of PBRs in improving the crop yields and water productivity through the regulation of physiological processes and plant–water relations has recently been elaborated through several reports (Khan et al., 2015; Srivastava et al., 2016; Wakchaure et al., 2016a&b). Though the most of PBRs have been tried under pot or controlled conditions, those reported for their viability include salicylic acid (Fayez and Bazaid, 2014); sodium benzoate (Beltrano et al., 1999; Kumar et al., 2014); thiourea (Bhunia et al., 2015; Wakchaure et al., 2016) and potassium nitrate (Gimeno et al., 2014). Nevertheless, there is general lack of information on the relative responses of PBRs under field conditions (Wakchaure et al., 2016a&b). So, the other objective was to evaluate the effectiveness of selected PBRs on yield, water productivity and post-harvest quality under variable water deficits in semi-arid Deccan Plateau of India.

1.3. Case studies and salient research findings

1.3.1. Responses of Wheat (HD−2189) to PBRs under varied water deficit

The interactive effect of irrigation regimes and PBRs on grain yield and water productivity of spring wheat (Triticum aeastivum L) were evaluated during three years (2012−15) using LSS (Fig.1). PBRs applied through exogenous sprays included: 10 mM thio-urea (TU), 10 uM salicylic acid (SA), 15 g L^{-1}potassium nitrate (KNO_3), 25 ppm gib-berellic acid (GA_3), 8 ppm ortho-silicic acid (OSA) at crown root initiation (CRI), flag leaf and seed milking stages and control (no PBR). Seven irrigation levels were generated through a line source sprinkler system (LSS) viz., application of irrigation water (IW) equalling 1.0, 0.85, 0.70, 0.55, 0.40, 0.25 and 0.10 times the CPE (cumulative open pan evaporation). The maximum yield obtained with PBRs varied between 4.11–

4.46 Mg ha^{-1} at IW: CPE 0.85 against 4.09 Mg ha^{-1}without PBR. While the yield decline equalled 0.35–0.42 Mg $ha^{-1}$for every 0.1 IW: CPE for PBRs against 0.43 Mg ha^{-1}without PBR (. The overall improvement in grain yield and total biomass with PBRs ranged between 5.9–20.6% and 4.8–15.3%, respectively. Specifically TU and SA showed a major role under medium (IW:CPE 0.40–0.69) and severe (0.10–0.39)stress conditions in terms of maintenance of leaf water content, modulating the stomatal opening and better water usage and thereby improved yield by 0.41–0.88 Mg ha^{-1} The maximum water productivity ranged between 1.20–1.35 kg m^{-3} with different PBR's while it was 1.18 kg m^{-3} without PBR and the latter could be achieved with 19–56% lesser irrigation water with PBRs. Overall conclusions are that the effects of deficit irrigation could be substantially enhanced in terms of grain yield and water productivity when used conjunctively with PBRs like TU and SA. Thus for integrating PBRs with supplemental irrigation, large scale testing is required for defining their economic spray schedules under water scarcity conditions (Wakchaure et al., 2016a).

Fig.1. Experimental setup and evaluation of PBRs using line source system (LSS)

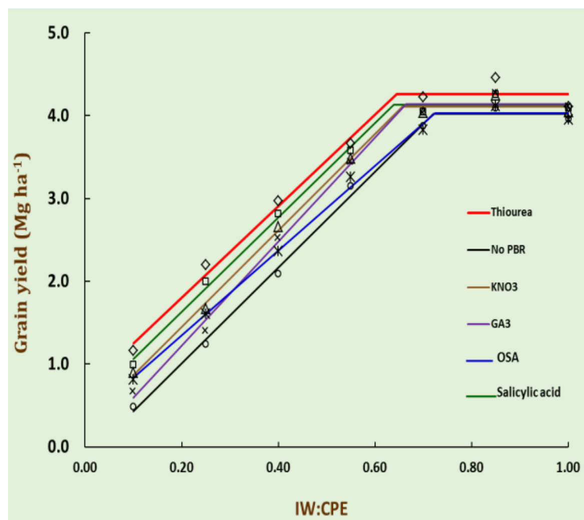

Fig.2 Relative response of PBRs to grain yield of wheat

1.3.2. Effect of plant bio-regulators on growth, yield and water production functions of sorghum [Sorghum bicolor (L.) Moench]

Effect of plant bio-regulators (PBRs) and supplemental irrigation on growth and grain yield of sorghum [*Sorghum bicolor* (L.) Moench] was evaluated (Fig.3) during two years (2015−2016). Exogenous application of PBR's included: 10 µM salicylic acid (SA), 100 mg L^{-1} sodium benzoate (SB), 500 ppm thiourea (TU), 1.5% potassium nitrate (KNO_3) at seedling elongation (20 DAS), reproductive (50 DAS) and panicle emergence (75 DAS) stages and control (no spray of PBR). The maximum grain yield (3.60–3.88 Mg ha^{-1}) was obtained at IW: CPE 0.80 and declined @ 0.43–0.49 Mg ha^{-1} for every 0.1 IW: CPE for PBRs and the corresponding values were 3.49 and 0.53 Mg ha^{-1} without PBR. The application of PBR's mitigated water stress and improved gain yield, straw yield and water productivity by 6.8–18.5%, 5.7−14.7% and 1.16−1.41 kg m^{-3}, respectively (Fig.4). SA was more effective under moderate (IW: CPE 0.79–0.50) while SB and TU were better under severe water deficits (IW: CPE 0.49–0.05). Thus SB and TU present viable option to reduce water use by 25.2-49.7% under the conditions of deficit irrigation (Wakchaure et al., 2016)

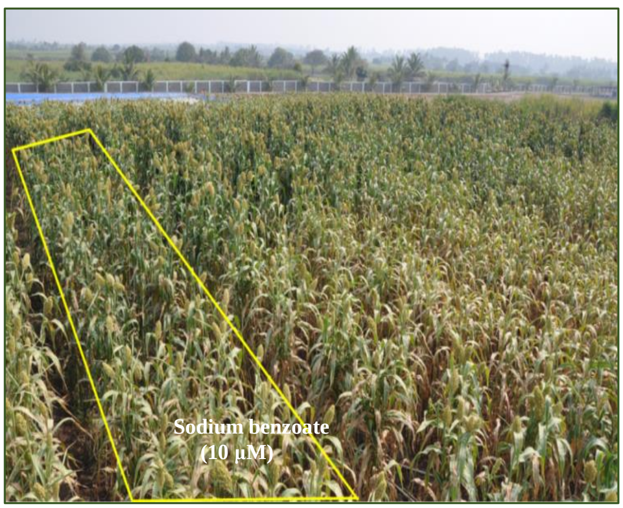

Fig.3. Sorghum response to PBRs

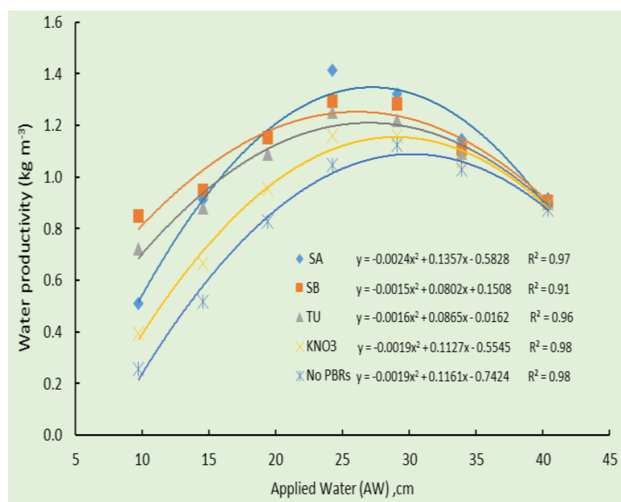

Fig.4. Water productivity as expressed as the function of PBR's at various quantities of applied water (AW)

1.3.3. Growth, bulb yield, water productivity and quality of onion (Alliumcepa L.) as affected by deficit irrigation regimes and exogenous application of plant bio–regulators

Effect of plant bio–regulators (PBRs) viz., potassium nitrate (KNO_3, 15 g L^{-1}), thio–urea (TU, 500 ppm), salicylic acid (SA, 10 µM), gibberellic acid (GA3, 25 ppm) and sodium benzoate (SB, 150 mg L−1) for two years (2015–17) under various levels of deficit irrigation created using line source sprinkler system (LSS) was evaluated in onion (Allium cepa L.) (Fig.5). The crop could sustain little water deficits and its bulb yield declined to 0.84, 0.66, 0.48, 0.35, 0.24 and 0.16 when irrigation water (IW) applied equalled 0.85, 0.70,0.55, 0.40, 0.25 and 0.10 times the pan evaporation (CPE) against maximum yield at full irrigation (IW:CPE1.00). Application of PBRs helped to mitigate the water stress through maintenance of leaf water content, modulating the canopy temperature and better water usage thereby improving average bulb yields by10.1–25%. Especially KNO_3 and TU were more effective under low to medium water deficits. The water productivity ranged between 7.78 and 9.61 with PBRs against 7.36 kg m^{-3} under control (Fig.6). The overall water saving was 18.3, 25.7, 48.4 and 63.8% with PBRs namely GA3, SA, TU and KNO_3, respectively. The marketable quality monitored in terms of bulb weight, geometric mean diameter and sphericity was significantly reduced with water deficits while it improved with PBRs. Among the other physicochemical and functional quality characteristics of the onion bulb, rehydration ratio, protein content, total soluble sugar, total phenolics content and pyruvic acid were lowered by water deficits. These were improved significantly with PBRs. Thus it was concluded that combining PBRs like KNO_3 and TU can further facilitate to implement deficit irrigation technology for sustaining productivity and quality of onion under water scarce conditions.

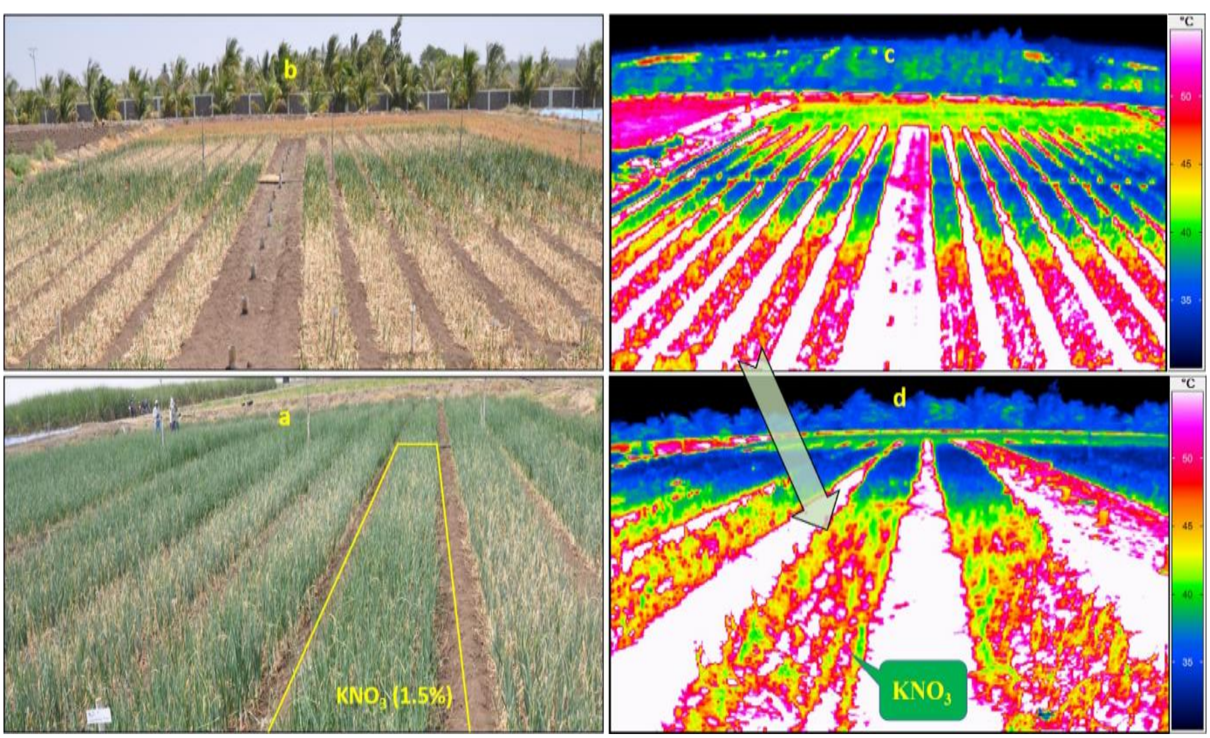

Fig.5. Onion responses to PBRs under varied irrigation water levels as depicted in IR image

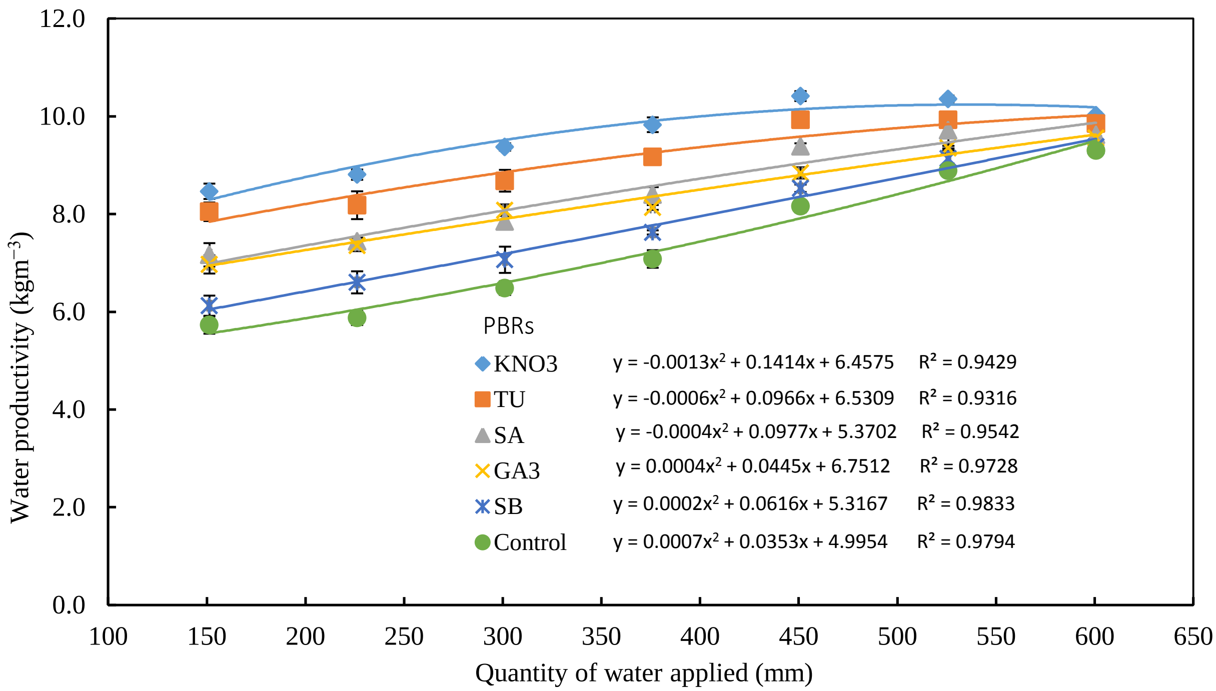

Fig.6. Water productivity as a function of PBRs and quantity of applied water (AW) during onion growth period

1.3.4. Exogenous application of PBRs for enhancing water productivity of eggplant (cv. Panchganaga)

Exogenous sprays of PBRs viz., 15 g L^{-1} potassium nitrate (KNO_3), 10 mM salicylic acid (SA), 500 ppm thiourea (TU) and 100 ml L^{-1} microbial biopolymer (BP) were applied at vegetative, flowering, fruit formation and development stages of eggplant. The interactive effect of PBRs, BP and supplemental irrigation on yield formation was evaluated using line source sprinkler system (LSS) at seven levels of irrigation water (IW) equalling to 0.90, 0.75, 0.60, 0.45, 0.30, 0.15 and 0.0 times of cumulative open pan evaporation (CPE). Application of PBRs and biopolymer significantly improved marketable yield and water productivity over control (Fig.7) . PBRs like salicylic acid (10 µM) at higher and KNO_3 (15 g L^{-1}) at lower irrigation levels present viable option to mitigate water stress and reduce water use by 50 per cent. Nutritional quality (TSS, protein contents and antioxidant enzymes activities) of brinjal enhanced significantly with PBR’s underwater deficits. Identified plants PBR’s like KNO_3, SA help to mitigate water stress and can help to boost the productions vis-a-vis profitability of onion under water scarcity conditions. Similarly use of microbial biopolymer can be better alternative for chemical PBRs for enhancing yield

Fig. 7. Relative response of PBRs and biopolymer to eggplants

1.4. Conclusions

- PBRs like thiourea (10 mM), sodium benzoate (100 mg L^{-1}), potassium nitrate and salicylic acid (10 µM) helped to mitigate water stress for wheat, sorghum, onion and eggplants, respectively
- The response of PBRs is highly specific environment conditions and varies with crop to crop
- The post-harvest quality measured in terms of physiochemical and functional characteristics (TSS, protein contents, total phenolics and antioxidant enzymes activities) of vegetable crops enhanced significantly with PBRs under water deficits
- Overall use of PBRs can help to boost the productions vis-a-vis profitability of crops under climate change in water scarce regions

1.5. References

1. Beltrano, J., Ronco, M.G., Montaldi, E.R., 1999. Drought stress syndrome in wheat isprovoked by ethylene evolution imbalance and reversed by rewatering, amino ethoxyvinyl glycine, or sodium benzoate. J. Plant Growth Regul. 18, 59–64.
2. Bhunia, R., Verma, I.M., Sahu, M.P., Sharma, N.C., Balai, K., 2015. Effect of drip irrigation and bioregulators on yield, economics and water use of fenugreek (Trigonella foenum-graecum). J. Spices Aromat. Crop 24 (2), 102–105.
3. Fayez, K.A., Bazaid, S.A., 2014. Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. J. Saudi Soc. Agric. Sci. 13, 45–55.
4. Gimeno, V., Diaz-Lopez, L., Simon-Grao, S., Martinez, V., Martinez-Nicolas, J.J., Garcia-Sanchez, F., 2014. Foliar potassium nitrate application improves the tolerance of Citrus macrophylla L. seedlings to drought conditions. Plant Physiol. Biochem. 83, 308–315.
5. Hanks, R.J., Sisson, D.V., Hurst, R.V., Hubbard, K.G. 1980. Statistical analysis of results from irrigation experiments using the line-source sprinkler system. Soil Science Society of America Journal 44(4), 886-888.
6. Khan, I.M.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid–induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 6, 462.
7. Kumar, B., Lamba, J.S., Dhaliwal, S.S., Sarlach, R.S., Ram, H., 2014. Exogenous application of bio-regulators improves grain yield and nutritional quality of forage cowpea (Vigna unguiculata). Int. J. Agric. Biol. 16 (4), 759–765.
8. Srivastava, A.K., Ratnakumar, P., Minhas, P.S., Suprasanna, P., 2016. Plant bioregulators for sustainable agriculture; integrating redox signaling as a possible unifying mechanism. Adv. Agron. 137, 237–278.
9. Wakchaure, G.C., Minhas, P.S., Ratnakumar, P., Choudhary, R.L., 2016a. Optimising supplemental irrigation for wheat (Triticum aestivum L.) and the impact of plant bio-regulators in a semi-arid region of Deccan Plateau in India. Agric.Water Manag. 172, 9–17.

10. Wakchaure, G.C., Minhas, P. S., Pasala, R.K., Choudhary, R. L., 2016b. Effect of bioregulators on growth, yield and water production functions of sorghum [Sorghum bicolor (L.) Moench]. *Agric Water Manage.*, 177, 138−145.

11. Wakchaure, G.C, Singh, N.P., Choudhary R. L., Meena, K.K., Minhas P.S., 2016. Effect of bioregulators on growth, yield and nutritional quality of onion (Allium cepa L.) under water stress in shallow basaltic soils, In: 2nd National Symposium on Edible Alliums: Challenges and Future strategies for sustainable production, November 7−9, 2016, Indian Society of Alliums and Beej Sheetal Bio-Science Foundation, Jalna, Maharashtra, CP−AB−48, pp. 238−239.

12. Wakchaure, G.C., Minhas, P.S., Meena, K. K., Singh, N.P., Hegade, P., Sorty , A.M. 2018. Growth, bulb yield, water productivity and quality of onion (Alliumcepa L.) as affected by deficit irrigation regimes and exogenousapplication of plant bio–regulators. *Agric Water Manage*, 199 (2018) 1–10.

Chapter- 2

Foldscope- An economically feasible research tool

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2.1. Introduction

The Foldscope is an origami-based optical microscope that can be assembled from a flat sheet of paper in less than 10 minutes. Manu Prakash, an associate professor of Bioengineering at Stanford University and his team developed these flat microscopes by combining principles of optical design with origami (Cybulski et al., 2014). The Foldscope performs most of the functions of a high-school lab microscope, but its parts cost less than a dollar. It consisted of a punched sheet of cardstock, a spherical glass lens, a light emitting diode and a diffuser panel, along with a watch battery that powers the LED. It can provide over 2,000 X magnification with submicron resolution, weighs less than two nickels (8.8 g), is small enough to fit in a pocket, requires no external power, and can survive being dropped from a 3-story building or stepped on by a person. Prakash Lab at Stanford University, USA in associated with Department of Biotechnology (DBT), Government of India brought the Foldscope to India to encourage curiosity in science.

This cost-effective and portable foldscope has good scope in agriculture field includes the study on viability and germination of pollens. These studies are important because the fate of pollen have a profound influence on crop yield as it has greater role in reproductive success. Our experimental results in garden plants, pulses and horticultural crops revealed that it can be used for pollen germination studies efficiently. Foldscope could differentiate the colour of viable and non viable pollens very efficiently in our pollen viability studies too (unpublished). Moreover it can be taken to field and observe the pollen viability at the field itself if we have lot many genotypes to observe at a time by engaging many people.

Here we explained the procedure to observe the pollen grains, its viability and germination by using fold scope.

2.2. Pollen viability studies

Materials: Forceps, Glass slides, cover slip, dropper, Triphenyl Tetrazolium Chloride stain (1% TTC in 5% sucrose (Oberle and Watson, 1953)), cellotape.

Method:

- Collect pollen grains in the morning time (8.00 AM-10 AM) depends on the crop.
- Dust pollen on the slides using a forceps or brush and mixed with a drop of the stain.
- Put cover slip on the specimen and seal it with cellotape.

- Insert the slide in the foldscope after 15 m of time and observe
- Select a field with a minimum of 10 pollens
- Record images using mobile camera
- Calculate the percent viability by counting total pollens and viable pollens

2.3. Pollen germination studies

Materials: Forceps, cavity glass slides, cover slip, dropper, germination medium (depends on crop), cellotape.

Method:

- Collect pollen grains as the above procedure.
- Dust pollen on the cavity slides using a forceps or brush and mixed with a drop of the medium.
- Put cover slip on the specimen and seal it with cellotape.
- Incubate the slides in a humid chamber at 22-25 °C.
- Insert the slide in the foldscope and observe
- Select a field with a minimum of 10 pollens
- Record images by using mobile camera
- Calculate the percent germination by counting total pollens and germinated pollen

Chapter- 3

Mushroom cultivation, processing and nutraceuticals: an agribusiness activity for enhancing the farmer's income

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3.1.Abstract

Meeting the food and nutritional security of rising population from the limited land resources is a big challenge in this vulnerable climate change area, which looks for new crop as source of food and nutrition. In this context, mushrooms find a favour which can be grown even by landless people, that too on waste material. Mushrooms is fleshy edible fungi, a rich source of good quality vegetative proteins, essential amino acids, minerals and vitamins with low calories neutraceutical food. Commercially very few mushrooms like button (*Agaricus bisporus*), oyster (*Pleurotus* spp.), milky (*Calocybe indica),* shiitake (*Lentinula edodes)* and paddy straw mushroom (*Volvariella volvacea*) are cultivated across the world. Most of the mushrooms, being high in moisture and delicate in texture, it cannot be stored for more than 24 hours at the ambient conditions prevailing in the tropics. Spoilage of mushrooms due to browning, wilting, liquefaction, loss of texture, aroma, flavour, etc., making it unsaleable. As far as processing concerned, several short term and long term post-harvest and preservation approaches like suitable harvest index, washing and grading, post-harvest handling, pre–cooling, packaging and packaging materials, modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), steeping and canning preservation, drying/dehydration technologies are available to extent the shelf life of the mushroom. Further mushroom processing can generates additional income to entrepreneurs through development of various medicinal and value added products. Overall on appropriate postharvest management practices for each type of mushroom should be designed on priority for enhancing the production and strengthen the marketing chain.

3.2.Rationale of mushroom cultivation

Apart from the important horticultural cash crop, mushrooms is potent neutraceutical food as it produced from recyclable organic wastes/agro-by-product. The land requirement is not pre–requisite in mushroom cultivation, as it is grown indoors in protected houses with environment control with intensive space utilization. Indoor commercial cultivation of mushrooms utilizes the vertical space and is regarded as the highest protein producer per unit area and time i.e. almost 100 times more than the conventional agriculture and animal husbandry. This hi-tech horticulture venture has a promising scope to meet the food shortages without undue pressure on land. Mushrooms, basically a form of fleshy edible fungi, are a rich source of good quality proteins, having most of the essential amino acids, minerals and vitamins with low calories. It also shown potential to produce numerous biologically active compounds that are typically associated with cell wall, and these have been suggested to contribute to enhancement of immunity and tumor retarding effects. Among the local communities, mushrooms may represent potential sources of antibacterial drugs, since in the early days, screening for antibiotics started with mushrooms and proved to be successful. Sixty antimicrobial compounds have been isolated from mushrooms; however, only the compounds from microscopic fungi have been present in the market as antibiotics until now. Mushrooms are rich in protein, dietary fiber, vitamins and minerals. Chemical compositions of different mushrooms are given in Table 1. In addition to these, mushroom is an excellent source of vitamins especially C and B (Folic acid, Thiamine, Riboflavin and Niacin). Minerals viz., potassium, sodium and phosphorous are higher in fruit bodies of the mushroom. It also contains other essential minerals (Cu, Zn, Mg) in traces but deficient in iron and calcium.

Table 1. Chemical compositions of different mushrooms (dry weight basis g/100g)

Mushroom	Carbohydrate	Fibre	Protein	Fat	Ash	Energy k cal
Agaricus bisporous	46.17	20.90	33.48	3.10	5.70	499
Pleurotus sajor-caju	63.40	48.60	19.23	2.70	6.32	412
Lentinula edodes	47.60	28.80	32.93	3.73	5.20	387
Pleurotus ostreatus	57.60	8.70	30.40	2.20	9.80	265
Vovarella volvaceae	54.80	5.50	37.50	2.60	1.10	305
Calocybe indica	64.26	3.40	17.69	4.10	7.43	391
Flammulina velutipes	73.10	3.70	17.60	1.90	7.40	378
Auricularia auricula	82.80	19.80	4.20	8.30	4.70	351

Currently mushroom farming is practiced in more than 100 countries and its production is increasing at an annual rate of 6-7%. In some developed countries of Europe and America, mushroom farming has attained the status of a high-tech industry with very high levels of mechanization and automation. China alone is reported to grow more than 20 different types of mushroom at commercial scale and mushroom cultivation has become China's sixth largest industry. Presently, three geographical regions– Europe, America and East Asia contribute to about 96% of world mushroom production. With the rise in the income level, the demand for mushrooms is bound to increase in other parts of the world as well. China has been producing mushrooms at very low costs with the help of seasonal growing, state subsidies and capturing the potential markets in the world with processed mushrooms at costs not remunerative to the growers in other mushroom producing countries. In India the mushroom production systems are mixed type i.e. both seasonal farming as well as high-tech industry. The concentrated areas of production in India are the temperate regions for the button mushroom, tropical and sub-tropical regions for oyster, milky, paddy straw and other tropical mushrooms. Two to three crops of button mushroom are grown seasonally in temperate regions with minor adjustments of temperature in the growing rooms; while one crop of button mushroom is raised in North Western plains of India seasonally. Oyster, paddy straw and milky mushrooms are grown seasonally in the tropical/sub-tropical areas from April to October. The areas where these mushrooms are popularly grown are Orissa, Maharashtra, Tamil Nadu, Kerala, Andhra Pradesh, Karnataka and North Eastern region of India. Thus different of edible and medicinal mushrooms can be cultivated round the in plains, medium elevated and hilly areas (Fig.1).

Button (A. bisporus)

Winter oyster mushroom

Shiitake mushroom

Flammulina velutipes

Pleurotus eous

Paddy straw mushroom

Milky mushroom

Reishi mushroom

Fig. 1 Different types of edible and medicinal mushrooms

3.3.Mushroom processing techniques

As far as mushroom processing technologies are concerned, it can be classified as short term (< 6 months) and long term (6-12 months) techniques depending on the storage period of mushrooms (Fig.2). These processing techniques could be applied for all types mushrooms with modification in processing operations. The sun drying of mushrooms is one of the simplest and oldest methods followed by the growers from the time immemorial. Due to the difficulties in drying of some of the mushrooms, new preservation technologies like canning, steeping, pickling, individual quick freezing, modified atmospheric packaging, mechanical and chemical drying (freeze drying, fluidized bed drying, batch type cabinet drying and osmotic drying), irradiation treatment, medicinal value added products of mushrooms have been developed to improve the shelf life and consumption of mushrooms.

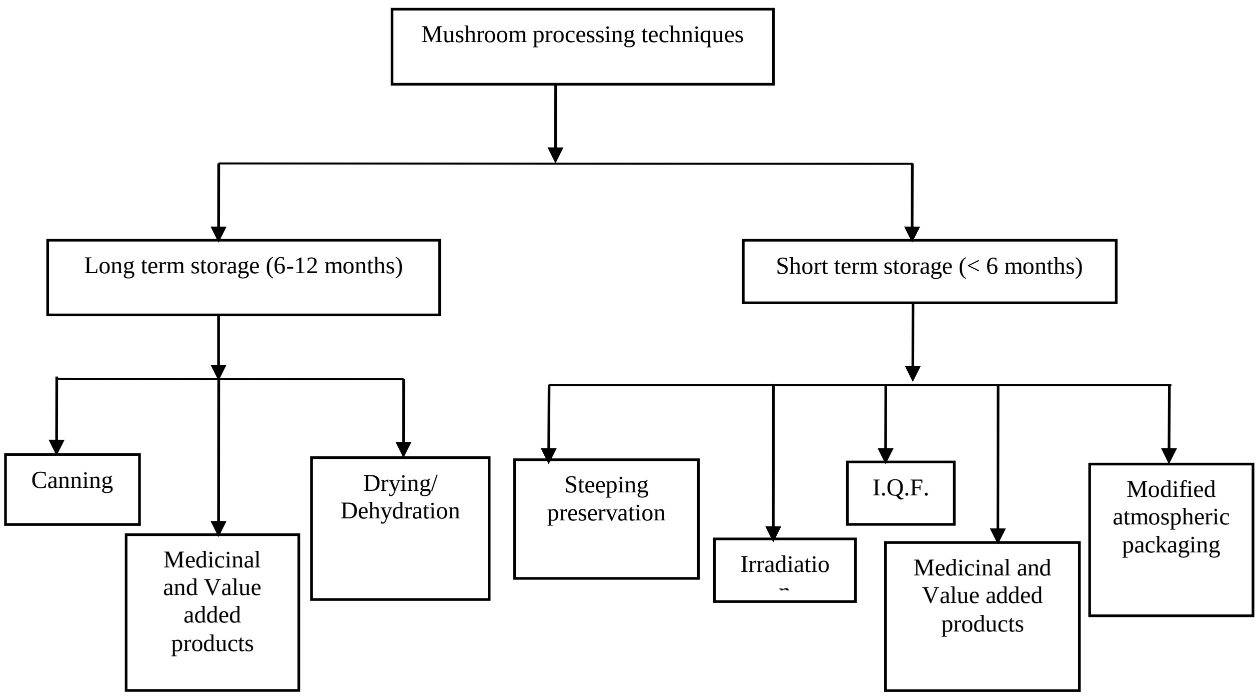


Fig. 2. Mushroom processing technologies

In recent years, rising demand of quality fresh vegetables including mushrooms has been reflected in the prices and marketability of the produce. The post-harvest measures are almost same for all types of fresh mushrooms (e.g. button, oyster, paddy straw and milky mushroom) with minute variation in processing operation depending upon their type. By following proper post-harvest measures, the shelf life and quality of harvested mushrooms can be extended. The general post-harvest practices followed for fresh mushrooms processing for marking is given in Fig. 3.

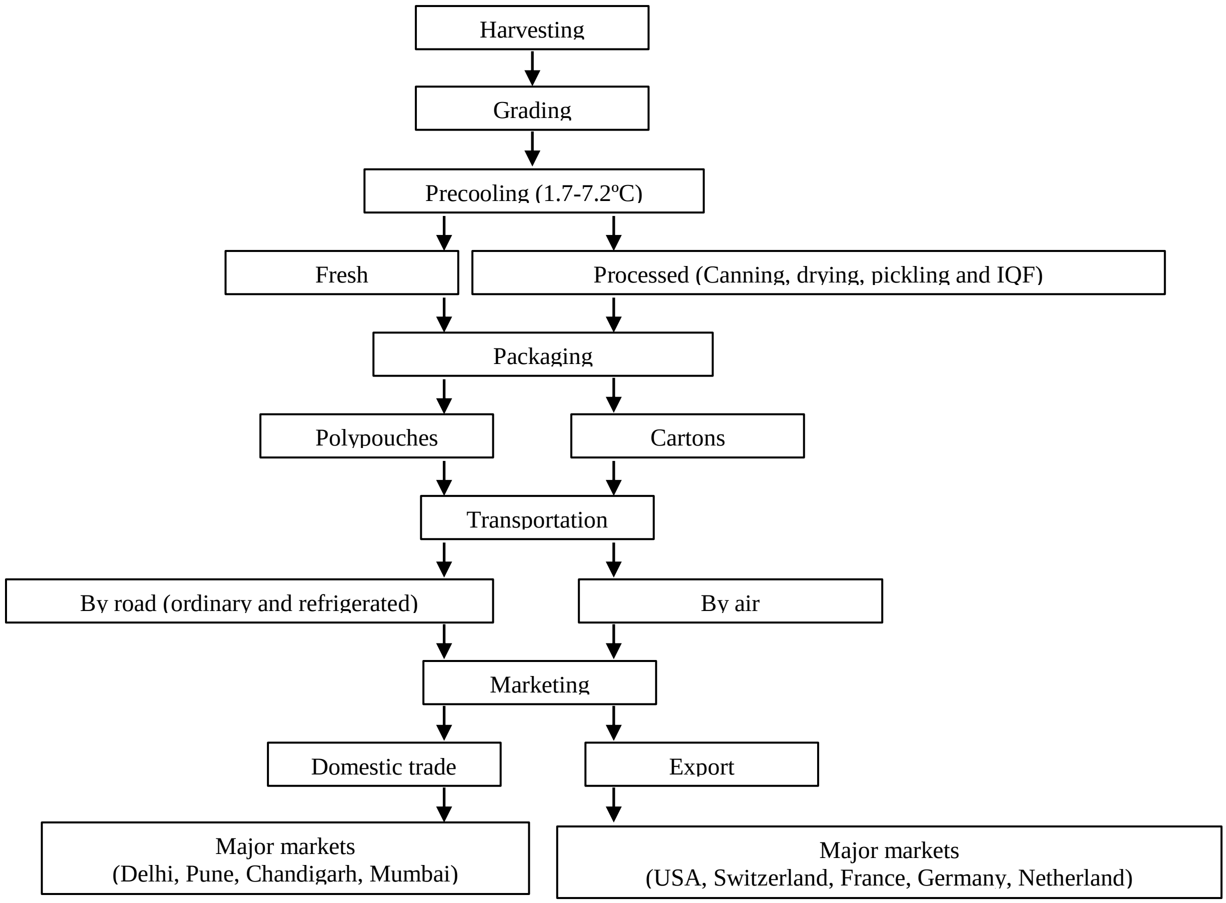


Fig. 3. General post-harvest practices for fresh mushroom marketing

3.4.Value added products of mushrooms

Worldwide, focus of mushroom industry is predominantly on trade of the fresh produce rather than the real value-addition. Domestically, almost entire mushrooms are consumed in fresh form while most of the export is in the preserved form (canned, steeped and dried). Current era is characterized by greater awareness about mushrooms and many other vegetables as a neutraceutical product with the demand for the readymade or ready-to-make food products. Mushrooms being delicacy and high moisture cannot be stored for more than 24 h at the ambient conditions of the subtropics/tropics which leads microbial spoilage of the product making it unsalable. Effective processing techniques will not only diminish the post-harvest losses but also result in greater remuneration to the growers as well as processors. Value can be added to the mushrooms at various levels, right from grading to the readymade snacks or the main-course item. Real value-added products in the Indian market are the mushrooms pickle and mushroom soup powder. Technologies for production of some other products like mushroom based biscuits, nuggets, preserve, noodles, *papad*, candies, cookies, ketchup, *murabba*, chips, chutney and readymade mushroom curry in retort pouches have been developed but are yet to be popularized. However, recently many private firms are coming forward for development of various value added products from mushrooms because of its increasing awareness. Attractive packaging of the value-added products is yet another area which may be called the secondary value-addition. While small growers may add value by grading and packaging, industry may go for the processed products for better returns as well as improvement in the demand, which shall have cascading positive effect on the production. Various mushroom value added products developed from mushrooms and their commercial packages are shown in Fig. 4.

Button mushroom soup powder

Oyster mushroom soup powder

Oyster mushroom pickle

Button mushroom pickle

Button mushroom biscuits

Oyster mushroom biscuits

Mushroom Nuggets

Mushroom *Murabba*

Mushroom Ketchup

Mushroom curry

Mushroom *Papad* Mushroom soup powder

Fig. 4. Various mushroom value added products

3.5. Mushrooms medicinal products

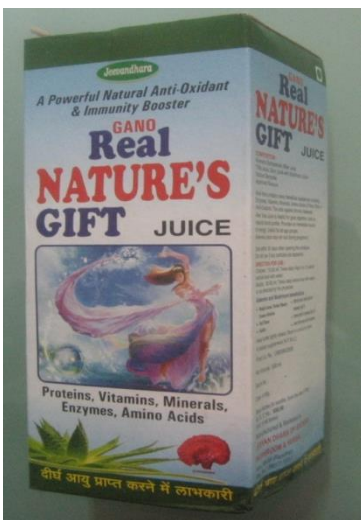
Mushrooms are considered to be a complete, health food and suitable for all age groups, child to aged people. The nutritional value of mushrooms is affected by numerous factors such as species, stage of development and environmental conditions. Mushrooms are an excellent source of proteins, dietary fiber, vitamins especially C, B (folic acid, thiamine, riboflavin and niacin), antioxidants, minerals viz., potassium, sodium and phosphorous. It also contains other essential minerals (Cu, Zn, Mg etc.) in traces but deficient in iron and calcium. Most of the edible fungi have been commonly known for their immense health benefits and extensively used in folk medicine. Specific biochemical compounds in mushrooms are responsible for improving human health in many ways. These bioactive compounds include polysaccharides, tri-terpenoids, low molecular weight proteins, glycoproteins and immune modulating compounds. Hence mushrooms have been shown to promote immune function; boost health; lower the risk of cancer; inhibit tumor growth; help balancing blood sugar; ward off viruses, bacteria, and fungi; reduce inflammation; and support the body's detoxification mechanisms. Increasing recognition of mushrooms in complementing conventional medicines is also well known for fighting many diseases. Medicinal functions of few important are given in the Table 2.

Table: 3 Medicinal mushrooms and their medicinal functions

Mushrooms	Compounds	Medicinal properties
Ganoderma lucidum	Ganoderic acid	Augments immune system
		Liver protection
	Beta-glucan	Antibiotic properties
		Inhibits cholesterol synthesis
Lentinula edodes	Eritadenine	Lower cholesterol
	Lentinan	Anti-cancer agent
A. bisporous	Lectins	Enhance insulin secretion
P. sajor-caju	Lovastatin	Lower cholesterol
G. frondosa	Polysaccharide Lectins	Increases insulin secretion

		Decrease blood glucose
Auricularia auricula	Acidic polysaccharides	Decrease blood glucose
Flammulina velutipes	Ergothioneine	Antioxidant
	Proflamin	Anti cancer activity
Trametes versicolor	Polysaccharide-K (Kresin)	Decrease immune system depression
Cordyceps sinensis	Cordycepin	Cure lung infections Hypoglycemic activity Cellular health properties Anti-depressant activity

Some extracted medicinal products viz., Reishi Gano, Corilus, real natures/ life revival juices derived from *Ganoderma Lucidum* lowers blood glucose, acts as antioxidants, immunity booster, cell regenerators and reduces gynecological problems in pregnant women's (Fig. 5). Similarly Cordyceps Cs a medicinal products derived from *Cordyceps sinensis* is used as energy and endurance.



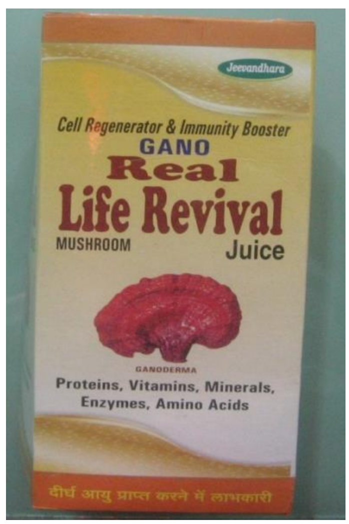


Fig. 5. *Ganoderma Lucidum* medicinal products

Mushrooms are not only source of nutritious food but also act as source of bioactive metabolites and prolific resource for drugs. Knowledge advancement in biochemistry, biotechnology and molecular biology boosts application of mushrooms in medical sciences.

From a holistic consideration, the edible mushrooms and its by-products may offer highly palatable, nutritious and healthy food besides its pharmacological benefits.

3.6.Futures of mushroom an agribusiness

Presently, India produces about 1.5 lakh metric tons mushrooms, which is about 3% of the world mushroom production. Because of high tech machinery with environmental control equipments imported and indigenous mushroom mechanization in 1990, India's mushrooms production were drastically increased and started export of canned mushrooms into international market of Europe and USA. Despite of huge biomass production and country's subtropical climatic conditions more favourable for cultivation of all kind of mushrooms, still India's mushrooms share in domestic and international market is almost negligible. Also for Indian mushrooms, China is the main competitor in international market. However, rising demand of fresh mushroom and improved prices in domestic as well as international market, India has a good chance to stands in mushroom business if we view the following Strength, Weaknesses, Opportunities and Threats (SWOT) analysis of Indian mushroom industry.

3.6.1. Strength

- Climatic diversification- suitable for all kind of mushroom cultivation
- Abundant raw material and man power coupled with government support
- Strategic location

3.6.2. Weaknesses

- Higher cost of finances, packaging materials, energy and transportation
- Poor quality of raw materials and higher cost machineries

3.6.3. Opportunities

- Decline production of other countries and breaking down of international trade barrier
- Increasing awareness and liking for mushrooms in domestic market
- Well adaptation of modern technologies and PHT management

3.6.4. Threats

- Competition from China and other countries
- Demand shifted towards the fresh consumption

3.7.Conclusions

Serious efforts in mushroom research for commercial cultivation especially for development of suitable low cost compost, disease resistant high yielding spawns, new varieties and educating the growers are in recent origin. However, still it felt to develop suitable mushroom harvesting techniques. For efficient production and post-harvest management / practices of mushrooms, growers at seasonal/small /household levels are needed to be trained. Special subsidy on mushroom transportation cost and provision for refrigerated vans in hilly areas helps to minimize losses during transport of highly perishable fresh mushrooms. In order to protect small farmers from the exploitation by middlemen, an efficient centralized marketing system with proper processing units may be needed set up surrounding the potential/ growing areas. Also the mushroom enterprise is a capital-intensive venture need financial assistance is available from NABARD, State Govt financial institutions, public sector banks with lower interest in India. Besides this, different incentives are also available from Department of Food Processing Industries, NHB, and State Govt. agencies engaged in agricultural development. In general, small seasonal growers are unable to avail of these facilities and therefore they may unite to form cooperatives or SHGs for better marketing and solving their problems. Overall, the India has to establish “A quality produce of India” for mushrooms in foreign market to exploit present declining trend of mushroom production in many other developing/ developed countries.

References

Singh, M. 2011. Mushroom Production: An Agribusiness Activity, Mushroom cultivation, marketing and consumption, publisher: Directorate of mushroom Research, Solan, Himachal Pradesh, India. pp.1-10.

Wakchaure, G.C., Meena K.K., P. Suresh Kumar., 2017. Recent advances in mushrooms processing. In: Kadam, D.M., Sharma, M. and Kaur, D. Vegetables processing and bioactive compounds. Pp.507-536.

Chapter- 4

Conservation Agriculture enhancing productivity and use efficiency in sugarcane cropping Systems

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4.1. Introduction

Across the world sugarcane is grown in about 27 million hectares in tropical and sub-tropical regions of more than 80 countries. Sugarcane is one of the important cash crops in India and it is cultivated over an area of 5.0 million hectare with average productivity of about 70 tonnes per hectare which is nearly 20-50 % lower than countries like Peru, Argentina, Australia and Brazil. India is the largest consumer and the second largest producer of sugar in the world after Brazil with 17 % share in world production. Due to its multi-purpose uses in different industries, the demand is increasing for the increased production of sugarcane and its sustainability. As per the estimate, by 2030 AD, India will require nearly 33 million tonnes of white sugar for domestic consumption alone while at present the annual sugar produced is about 25 million tonnes. To bridge this gap, national sugarcane production and sugar recovery need to be enhanced with minimum inputs and restricted use of water particularly in the context of global climate change. In past much emphasis has been given on input intensive conventional crop production practices *i.e.* maximum tillage, clean cultivation, mono-cropping/ fixed crop rotation: exclusion of legumes, imbalanced fertilizer use, little use of organics, indiscriminate use of irrigation water etc. These practices has resulted in declined ground water table, poor resource-use efficiency, multi-nutrients deficiencies and deteriorating soil health, high production cost, diminishing farm profitability, degradation of natural resources, air and ground water pollution and so on. India is one of the top sugar producers in the world, but it did not have comparative advantage and trade competiveness in the international market because of high cost of cultivation, low crop productivity and poor sugar recovery. Further, the issue of sustainability has also gathered increased relevance and with the advent of climate changes that impact conditions for optimum production and profitability from the crop. These situations, therefore warrants to opt alternative crop production systems for reducing cost and making sugarcane cultivation climate resilient and sustainable such as “Conservation Agriculture” system.

4.2. Conservation Agriculture System

Retaining crop residues as surface mulch, together with minimum soil disturbance and crop rotation and association, forms the basis of conservation agriculture (Kassam et al., 2009). Conservation Agriculture (CA) includes the basic elements of such a sustainable production system, increasing productivity and production while reducing the need for external inputs and the environmental footprint of farming. CA improves the delivery by agriculture of ecosystem services such as water resources, biodiversity and the mitigation of climate change while strengthening the ecological foundation of cropping systems to also adapt to changing climates. Conservation agriculture is practiced on about 156 million ha worldwide. The Argentina, Australia, Breazil, Canada and America are holding around 90 % of the total area under CA. The Indo-Gangetic-Plains include four countries in South Asia, India, Pakistan, Nepal and Bangladesh are occupying around 5.0 million ha area under no-tillage system. In India, it is practiced in more than 2.0 million ha area mainly in rice-wheat cropping system of the Indo-Gangetic-Plains.

4.2.1. Major Benefits of CA

(Kassam *et al.,* 2009; Derpsch *et al.,* 2010; Jat *et al.,* 2013)

- Build-up of organic carbon and arrest decline in factor productivity
- Saving top fertilized soil from erosion
- Enhance nutrient-use efficiency by creating favorable environment for microflora and fauna
- Reduce water requirement of crops by cutting evaporation
- Check non-point pollution of nearby water bodies
- Help in sequestering GHG in the soil
- Improve biological activity and diversity
- Overall – reduce costs & energy use, efficient input use, stable yields, better use of natural resources

These benefits of CA are needed to be reaped in other input intensive cropping systems such as sugarcane system.

4.2.2. Why CA need to be adopted in sugarcane cropping system?

High energy requirement

- Sugarcane cultivation is labour intensive (10-15% to total energy requirement), which increased the cost of cultivation by 25-30 %.
- Seed bed preparation and planting account for more than 50% of operational energy requirement (Srivastava, 2003).

  Thus, the energy requirement of the crop could be reduced substantially by adoption of reduced/ zero tillage practices.

High water requirement

- Sugarcane is utilizes more than 5 % of irrigation resources of the country.
- It is a water guzzling crop requires 600 to 2000 mm of water annually for one hectare of crop area.
- It has been estimated that to produce one kg of sugar 60 to 90 litres of water is required.

It has been observed that due to unchecked exploitation of groundwater and resulting poor output of tube-wells the crop suffers badly due to moisture stress during summer months. As estimated only 35% of sugarcane area gets optimum irrigation whereas remaining 65% area is under sub-optimal irrigation and un-irrigated conditions. Irrigation water scarcity in Maharashtra has severely affected the cane productivity and options are being explored to replace sugarcane by a less water requiring crop like sugar-beet. The situation therefore warrants for adoption of conservation agriculture systems that reduce the water requirement of the crop by cutting the evaporation losses and conserving the moisture in soil.

Nutrient exhaustive crop

Sugarcane is a nutrient exhaustive crop, Sugarcane crop to yield 100 t/ha removes 208, 53, 280, 3.4, 1.2, 0.6 and 0.2 kg/ha of N, P, K, Fe, Mn, Zn and Cu respectively from soil (Yadav and Dey, 1998).

Replenishing the soil with these nutrients in a judicious and balanced way is imperative to ward-off the ill effects of high analysis fertilizer based approach of nutrient management. An integrated approach involving crop residue recycling, bio-fertilizers and green manuring, as envisaged under conservation agriculture holds promise for not only the build of soil organic carbon but also maintaining the soil ecological balance.

4.3. Challenges in Implementation of CA in Sugarcane Systems

About 15-20 t ha^{-1} trash is left after sugarcane harvest which is usually burnt because of constraints for fertiliser placement and other intercultural operations (Fig. 1).

Fig. 1. Photograph showing the open burning of sugarcane residue (trash).

Trash burning results in loss of organic carbon, plant nutrients such as N and S (Hemwong et al. 2009), soil biota besides the environmental and health hazards due to release of soot particles, smoke and greenhouse gases (Arbex et al. 2007). Several benefits has been reported due to retention of sugarcane trash on the soil surface over the burning such as it prevent weed growth and soil erosion, conserve soil moisture, improve soil structure, increase soil organic matter, cation exchange capacity, nutrient levels (Basanta *et al*., 2003), carbon sequestration (Hemwong *et al*., 2008) and nutrients recycling in the agro ecosystems (Fortes *et al*., 2012) and improve the environmental quality by reducing the emissions of GHGs due to residue burning. Nevertheless, the practice of surface retention of trash is not becoming popular due to non-availability of appropriate machinery particularly for placement of fertilizers under surface retained trash conditions.

4.4. Opportunities/ scope of conservation agriculture in sugarcane system

Require less tillage operations

- A cropping system based on sugarcane generally occupies the field for more than 2 years duration and comprises one plant, at least one ratoon and other crops in sequence.
- Often sugarcane plant and ratoon crops remain in field for more than half of the total period of a particular crop rotation. This indicates that sugarcane based cropping systems automatically do away with the requirement of frequent land preparations (primary tillage), normally done under other cropping systems.

4.5. Plenty availability of crop residue

- Maintaining the soil cover/ surface mulch with crop residues is an integral component of the conservation agriculture system. However, sometimes it is very difficult to retain the crop residues in the field particularly in the rainfed areas where crop residues is usually used as source of feed for animals. In contrast to this, availability of the crop residue in sugarcane based cropping systems is plenty.
- In India, sugarcane is the third largest crop residue (trash) producing crop after rice and wheat, though it occupies only around 5.0 million ha area (Table 1; Yadav and Srivastava, 2005).
- Trash production from sugarcane varies with the variety, season of planting, duration of the crop and climatic conditions. However, it generates on an average 10-15 tonnes trash/ ha. This could be used directly in the ratoon crop which is occupying around 50 % area of the of the sugarcane, while indirectly could be recycled in either plant crop or other crops in the sequence.

Table 1. Estimates of the crop residue yield and realizable plant nutrient potential from the residues of principal crops in India.

Crop	Residue yield (‘000 tonnes)	Nutrient concentration (%)			Total N, P, K potential (‘000 tonnes)
		N	P	K	
Rice	80744	0.61	0.09	1.15	1493.8
Wheat	44987	0.48	0.07	0.98	688.3
Sorghum	11163	0.52	0.12	1.21	216.2
Maize	6219	0.58	0.09	1.25	119.4
Pearl millet	8283	0.45	0.07	0.95	121.6
Barley	3180	0.52	0.08	1.25	58.8
Sugarcane	15645	0.45	0.08	1.2	270.7
Potato	5062	0.52	0.09	0.85	73.9
Groundnut	9580	1.65	0.12	1.25	277.3

4.6. Provides ample scope for crop diversification/intensification

- Sugarcane is a widely spaced crop (1.0-1.5 m distance between two rows), provide ample scope for maintaining surface cover either by growing of inter-crops or through mulching with trash for the longer durations.
- Intercropping of *rabi* crops like potato, mustard, wheat, pea, coriander, lentil, garlic and other winter vegetables has been found more remunerative as compared to autumn sugarcane grown alone or *rabi* crops followed by cane in sequence.

- ❖ Crops such as urd, mungbean, and cowpea that mature within a short period (75 to 90 days) are most compatible intercrops with spring cane and sometimes ratoons.

Thus, sugarcane is having the scope to comply with all the three basic principles of conservation agriculture namely minimum soil disturbance, permanent soil cover and crop rotations.

4.7. Availability of CA machines

The adoption of conservation agriculture on large scale in rice-wheat cropping system in the Indo-Gangetic Plains of India is due to development/ availability of appropriate CA machineries. Therefore, to accelerate the implementation of CA in sugarcane based cropping system, top priority should be given on the development of suitable CA machines. The development of trash shredder/ chopper/ cutter has brought the revolution in handling the sugarcane trash and opened the avenues for implementation of CA in sugarcane based cropping systems (Fig. 2). The trash cutter chopped the trash in small pieces and left on the soil surface, thus only suitable for one operation i.e. trash chopping. These machines are becoming popular (Sri Escorts: Sreenath Sugarcane Trash Cutting Machine, Tamil Nadu; Guru

Fig. 2. Photograph showing working of trash cutter

Agro Industries, Haryana and Maharashtra) but those like 'Happy Seeder" are not available where drilling of fertilizers is possible with surface retained straw in rice-wheat cropping system. Recently, efforts have been made in this line and a multi-purpose SORF machine has been developed (Choudhary et al., 2017). The SORF machine performs stubble shaving, off-barring, root pruning and placement of basal doses of fertilizers while retaining the chopped trash on soil surface in a single run (Fig. 3). Choudhary et al. (2017) have reported that with the use of SORF, cane yield and nitrogen uptake efficiency improved by up to 22 and 11 %, respectively over the over the trash burning and broadcast application of fertilizers. Thus, SORF machine has the potential to encourage the farmers to adopt conservation agriculture in sugarcane based cropping systems. However, there is need to develop a machine that could perform all the important operation like trash chopping, stubble shaving, off-barring, root pruning, placement of fertilizers and sowing of crops in a single run for reducing cost and energy requirements.

Fig. 3. Photograph showing working of multi-purpose SORF machine

(Source: Choudhary *et al.*, 2017)

4.8. Energy use-efficiency

Farm mechanization plays a vital role for the success of CA in different agro-ecologies and socio-economic farming groups. It ensures timeliness, precision and quality of field operations; reduces production cost; saves labour; reduces weather risk in changing climatic scenarios; improves productivity, environmental quality, sustainability and generates rural employment on on-farm and off-farm activities (Ladha et al. 2009). Reduced labour and machinery costs are economic considerations that are frequently given as additional reasons to use CA practices. Adopting conservation agriculture techniques is a holistic approach for management of soil and water resources, and improving efficiency and productivity per unit of C-based energy consumed. Compared to intensive tilled conventional rice-wheat system, ZT systems require much lesser energy and gives higher energy output; input ratio as well as higher system productivity (Gangwar et al. 2006). For example, continuous ZT with effective weed management using recommended herbicide + 1 hand weeding was more remunerative and energy efficient in Vertisols of Central India, and it was suggested that conventional till-based rice–wheat system could be replaced with zero-till-based crop establishment method with effective weed control measures to save labor and energy. Similarly, low-cost of cultivation, minimum energy usage, higher water productivity, higher net returns and enhanced energy input: output ratio were reported in ZT maize-wheat cropping system (Ram et al. 2010).

4.9. Soil health

Soil health denotes a state of dynamic equilibrium between flora and fauna and their surrounding soil environment in which all the metabolic activities of the former proceed optimally without any hindrance, stress or impedance from the latter. A healthy soil would ensure proper retention and release of nutrients and water, promote and sustain root growth, maintain soil biotic habitat, respond to management and resist degradation. Soil erosion, organic matter decline, compaction and salinization resulting from the CT based agriculture are the major threats to soil health. Conservation agriculture, which prescribes ZT coupled with crop residue mulching and diversified crop rotation, has come forward as a sustainable management system that could revert physical soil degradation in resource poor farms across very different agro-ecological conditions (FAO 2012). Intensive tillage accelerates soil organic carbon (SOC) loss as CO_2 as a result of physical disruption and enhanced biological oxidation. It is estimated that agriculture has contributed 25% of the historical human-made emissions of CO 2during the past two centuries. Loss of SOC could significantly be reduced by shifting from CT to ZT and other low-disturbance techniques. That's why conservation tillage systems are proposed as a way of achieving SOC sequestration, as relatively higher SOC in the plough layer is noticed under ZT than in CT. It was projected that the conversion of a conventional system to conservation tillage could mitigate approximately 20% of the USA agricultural greenhouse gas emissions (Del Grosso et al. 2005), and could result in a 0.50 MT ha^{-1} yr^{-1} C sequestration rate. The principle of maintaining a permanent soil covers either by planting a cover crop or by using crop residues eventually increases the amount of organic matter and available organic carbon in the soil. The benefit of crop residue recycling is higher when used as mulch on ZT soil than its incorporation under CT system. For example, crop residue treatment in ZT soils showed significantly higher amount of SOC than other treatment combinations in the top 15 cm soil depths (Table 5). Crop residue served as a source of carbon especially in upper soil layers. Zero-tillage practice minimizes exposure of

SOC from oxidation, and thus ensuring higher SOC content in surface soils of ZT with crop residue application.

Table 2. Effects of tillage and residue treatments on the SOC content (Ghimire et al. 2008)

Soil Depth (cm)	Soil Organic carbon (kg m^{-3})				LSD
	CT		ZT		
	M_0	M_1	M_0	M_1	
0-5	11.01	12.12	12.73	14.23	1.72
5-10	8.53	10.83	10.08	10.94	1.72
10-15	7.13	9.26	10.11	8.06	1.72
15-30	4.63	5.73	5.80	4.82	1.72
30-50	4.43	4.90	4.69	3.99	1.72
0-50	7.15	8.57	7.81	8.68	0.77

M_o: No crop residue, M_1: crop residue @ 4 tonnes ha^{-1} for each crop in the rotation

4.10. Conclusion

Conservation agricultural systems are more climate resilient, sustainable, profitable and have low greenhouse gases emissions. Sugarcane has wide potential to adopt conservation agriculture principles and it is the best option we have today for the Sustainable Production of crops. Just, we need to learn how to adapt and apply the principles to farmer conditions and circumstances and sustain the soil health as well as increase the efficiency of inputs. However, adequate and very specific mechanization is needed for its successful implementation and harnessing the potential benefits.

4.11. References:

1. Arbex, M.A., Martins, L.C., de Oliveira, R.C., Pereira, L.A.A., Arbex, F.F., Cancado, J.E.D., Saldiva, P.H.N. and Braga, A.L.F. 2007. Air pollution from biomass burning and asthma hospital admissions in a sugar cane plantation area in Brazil. *Journal of Epidemiology and Community Health* 61: 395–400.
2. Basanta, M.V., Dourado-Neto, D., Reidchardt, K., Bacchi, O.O.S., Oliveira, J.C.M., Trivelin, P.C.O., Timm, L.C., Tominaga, T.T., Correchel, V., Cassaro, F.A.M., Pires, L.F. and de Macedo, J.R. 2003. Management effects on nitrogen recovery in a sugarcane crop grown in Brazil. *Geoderma* 116: 235–248.

3. Choudhary, R.L., Wakchaure, G.C., Minhas, P.S. and Singh, A.K. 2017. Response of Ratoon Sugarcane to Stubble Shaving, Off-barring, Root Pruning and Band Placement of Basal Fertilisers with a Multi-purpose Drill Machine. Sugar Tech 19(1):33–40.
4. Del Grosso SJ, Mosier AR, Parton WJ, Ojima DS (2005) DAYCENT model analysis of past and contemporary soil $NO_2$and net greenhouse gas flux for major crops in the USA. Soil and Tillage Research 83: 9–24
5. Derpsch, R., Friedrich, T., Kassam, A. and Hongwen, L. 2010. Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural and Biological Engineering*. Int J Agric & Biol Eng, 3(1): 1-25.
6. FAO (2012) Food and Agriculture Organization of the United Nations, 2012. Available online at http://www.fao.org/ag/ca/6c.html
7. Fortes, C., Trivelin, P.C.O. and Vitti, A.C. 2012. Long-term decomposition of sugarcane harvest residues in Sao Paulo state, Brazil. *Biomass and Bioenergy* 42: 189–198
8. Ghimire R, Shah SC, Dahal KR, Duxbury JM, Lauren JG (2008) Soil organic carbon sequestration by tillage and crop residue management in rice-wheat cropping system of Nepal. Journal of Institute of Agriculture and Animal Sciences 29: 21–26
9. Gangwar KS, Singh KK, Sharma SK, Tomar OK (2006) Alternative tillage and crop residue management in wheat after rice in sandy loam soils of Indo-Gangetic Plains. Soil and Tillage Research 88: 242–252
10. Hemwong, S., Cadisch, G., Toomsan, B., Limpinuntana, V., Vityakon, P. and Patanothai, A. 2008. Dynamics of residue decomposition and N_2 fixation of grain legumes upon sugarcane residue retention as an alternative to burning. *Soil & Tillage Research* 99: 84–97.
11. Hemwong, S., Toomsan, B., Cadisch, G., Limpinuntana, V., Vityakon, P. and Patanothai, A. 2009. Sugarcane residue management and grain legume crop effects on N dynamics, N losses and growth of sugarcane. *Nutrient Cycling in Agroecosystems* 83: 135–151.
12. Jat, M.L., Gathala, M.K., Saharawat, Y.S. Tetarwal, J.P., Guptaa, R. and Yadvinder-Singh. 2013. Double no-till and permanent raised beds in maize–wheat rotation of north-western Indo-Gangetic plains of India: Effects on crop yields, water productivity, profitability and soil physical properties. *Field Crops Research* 149: 291–299.

13. Kassam, A., Friedrich, T., Shaxson, F. and Pretty, J. 2009. The spread of Conservation Agriculture: justification, sustainability and uptake. *Int. J. Agric. Sustain.* 7: 292–320.

14. Ladha J. K, Kumar V, Alam MM, Sharma S, Gathala M, Chandna P, Saharawat YS, Balasubramanian V (2009) Integrating crop and resource management technologies for enhanced productivity, profitability, and sustainability of the rice– wheat system in South Asia. (In) Ladha JK, Singh Y, Erenstein O, Hardy B (Eds.) Integrated Crop and Resource Management in the Rice–Wheat System of South Asia, IRRI, Los Banos, Philippines, pp. 69–108

15. Srivastava, A.C. 2003. Energy savings through reduced tillage and trash mulching in sugarcane *Sugar Crops Newsletter* 8(1): 2-3.

16. Ram H, Kler DS, Singh Y, Kumar K (2010) Productivity of maize (*Zea mays*) – wheat (*Triticum aestivum*) system under different tillage and crop establishment practices. Indian Journal of Agronomy 55: 185-190

17. Yadav, R.L., Srivastava, T.K., 2005. Conservation Agriculture and Opportunities for Sugarcane Based Cropping Systems, in: Abrol, I.P., Gupta, R.K., Malik, R.K. (Eds.), Conservation Agriculture – Status and Prospects. Centre for Advancement of Sustainable Agriculture, New Delhi pp. 242.

18. Yadav, D.V. and Dey, P. 1998. Integrated nutrient management based on systems approach. Applied Engineering in Agriculture 19(1): 40-45

Chapter- 5

Microbes Mediated abiotic stress management in crop plants

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5.1. Introduction

Plants come across with a variety of abiotic elements under regularly altering environmental situations. Most of the extreme abiotic factors are detrimental to plants, which significantly influence the growth, development, and productivity. All such abiotic factors are cumulatively termed as abiotic stresses. Prominent abiotic stressors include salinity, drought, low/high light, heavy metals, acidity, sodicity, wind, heat, frost, flood (submergence), etc. Plants have indigenous stress-responsive mechanisms, however, there exhibit inherent physical, morphological and molecular restrictions that limit the capability to respond to diverse abiotic stresses Abiotic environmental conditions such as drought, salt, heavy metals, and temperature can induce increased production of ROS by limiting the ability of a plant to utilize light energy through photosynthesis. Reactive Oxygen Species (ROS) are constantly produced by plants as a consequence of aerobic metabolism during their development or in response to abiotic stresses. ROS includes singlet oxygen (1O2), superoxide anion (O2.–), hydrogen peroxide (H2O2), and hydroxyl radical (OH.) species. These molecules are highly toxic, since they are able to modify all the primary constituents of the cell such as lipids, DNA, carbohydrates, and proteins. Soil salinity leads to accumulation of high concentration of sodium (Na^+), chloride (Cl^-), and sulfate (SO_4^{2-}) ions in plant cells, inactivating enzymes and inhibiting protein synthesis. The resultant condition reduces the rate of photosynthesis and in turn plant growth

Plants continuously interact with various kinds of microbes from soil microbial communities of the extreme pool of biological diversity in the nature. The seeds and roots exterior provide ideal habitat for microbial growth and development. Beneficial plant–microbe and microbiome interactions might characterize a promising sustainable solution to improve agricultural production both qualitatively and quantitatively. Plants establish association with a vast diversity of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB), which can enhance both the plant health and productivity. The beneficial microbial communities generally interact with plant by two strategies: first being the plant growth promotion by producing phytohormones, VOCs, cytokinins, siderophores, etc. and, second one, to work as biocontrol agents against phytopathogens. Both the strategies result in improved health of plants, enhanced immunity against phytopathogens, and efficient tolerance to biotic and abiotic stresses.

5.2. Microbes Mediated Alleviation of Abiotic Stress

Many PGPR impart good effects during abiotic stress condition by direct and indirect mechanism such as biofilm formation, chemotaxis, siderophore, EPS, and indol acetic acid (IAA) production, and through 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity Use of beneficial microbes as an integral component of agricultural practice is a promising technology which should be endorsed to enhance crop productivity in a sustainable and environmentally friendly manner under environmental stress conditions.

5.2.1. ACC Deaminase Production

Legumes when inoculated with mixed cultures of rhizobium and ACC deaminase-positive PGPR stimulates nodulation through inhibition of ethylene biosynthesis thereby enhancing nodulation and nitrogen fixation. A few examples include early growth and enhancement of nodulation in *Glycine max* by ACC deaminase producing rhizobacteria; and improved nodulation in *Pisum sativum* by ACC deaminase producing *Rhizobium leguminosarum* bv. Viciae 128C53K (Cattelan et al. 1999). An increase in lateral root density and length as well as root hair density and length (59% and 200%), respectively was observed in drought-stressed wheat plants when inoculated with 1- aminocyclopropane-1-carboxylate ACC deaminase, and IAA producing *Bacillus thuringiensis* (Timmusk et al., 2014). Rhizobacteria isolated from rice rhizosphere containing ACC deaminase were also found effective in enhancing salt tolerance and consequently improving the growth and development of rice plants under salt-stress conditions (Bal et al. 2013)

5.2.2. Microbial Volatile Organic Compounds (MVOCs)

Microbial volatile organic compounds (MVOCs) are synthesized by numerous microorganisms ranging from bacteria to fungi. MVOCs have prospective as promising replacements to harmful insecticides, fungicides, and bactericides as well as genetic modification. MVOCs are complex mixture of low molecular weight compound and are vital infochemicals mediating essential communication systems in all kingdoms of life. MVOCs are involved in plant rhizospheric processes like competence, pathogenesis, and symbiosis and also work as quorum-sensing signal molecules for both microbial growth and root development. Rhizospheric bacterial strains release several VOCs that can potentially control both the plant growth promotion and root-system development (Gutierrez-Luna et al. 2010) *Fusarium oxysporum* MSA 35 produces VOC β-caryophyllene which induces shoot length, root length, and fresh weight of lettuce seedlings (Minerdi et al. 2011). More than over 1000 microbial volatiles are described and documented in a distinct database for microbial VOCs called mVOC1 (Lemfack et al. 2014). The numbers of volatiles reported are very low as compared to the microbial diversity present in soil; there is need of more study about MVOCs for application in agriculture against the abiotic stress.

5.2.3. Microbial Derivatives of Plant Growth Hormones (PGHs)

Plant growth hormones synthesized by microbes are well studied. Hormones like auxins and cytokinins are known to play key role in the plant-microbe signaling as well as regulation of the plants' growth. Auxins and cytokinesis are also involved in the regulation of the symbiotic nitrogen fixation of rhizobium-plant interaction. The influence of cytokinins in the initiation of nodule organogenesis was also well studied in diverse legumes, where exogenous applications of cytokinins successfully induced amyloplast accumulation, cortical cell divisions, and the expression of initial nodulation indicators. Commonly majority of the auxin-producing bacteria produce IAA, while some of the other bacteria produce GA3 and other derivatives. IAA also has the ability to influence the gene expression in microbes; thus IAA contributes central role in plant-microbe interaction. A recent study demonstrated that *Pseudomonas* sp., *Rhizobium* sp., *Enterobacter* sp., *Pantoea* sp., *Marinobacterium* sp., *Acinetobacter* sp., and *Sinorhizobium* sp., able to produce IAA, have significant influence on germination and seedling growth in wheat under saline condition. *Pseudomonas* sp. and *Acinetobacter* sp. were also described to enhance production of IAA in barley and oats, in salinity stress conditions. Saharan and Nehra et al. (2011) demonstrated that *Azospirillum, Azotobacter, Pseudomonas* increased the plant growth and yield by a variety of mechanisms, among those, one was the production of phytohormones. Phytohormones are the principal constituent of protein fluctuations and can increase the yield and quality of oilseed crops (Lone et al. 2005)

5.2.4. Nutrient availability

The plant depends on bioavailability of both macro- and micronutrients. Most of the times, abiotic stress conditions alters the soil properties; for instance, conditions like salinity, acidity, alkalinity, metals contamination, etc. induce extreme changes in the physicochemical characteristics of soil. This badly affects the bioavailability of nutrients from the soil. The altered soil pH can potentially induce chemical fluctuations amongst soil nutrients, thereby making them biologically unavailable. Such nutrients though are available in the soil, they become biologically unavailable, to which plants fail to absorb, which directly influence the plant growth.

The rhizosphere microbes produce relatively large quantities of siderophores which actively chelate Fe+++ and facilitate its bioavailability. Many siderophore-producing microbes have been shown to actively enhance iron fulfillment to the plant under iron-starved soils (Supanekar et al. 2013). Fixation of atmospheric nitrogen by rhizosphere microbes is another important task. Many microbial strains have been described in literature for their exceptional ability to fix atmospheric nitrogen under abiotic stress environment (Bianco and Defez 2009).The use of such resilient nitrogen fixers under the influence of stressors like salinity and altered pH conditions can ensure a sustainable supply of nitrogen, as addition of chemical fertilizers may worsen the situation over prolonged period. Soil Microbial communities perform a central role in the carbon sequestration process by transforming plant residues into smaller carbon molecules that are more likely to be protected and sequestered. The microbial contribution to soil C storage is directly related to microbial community dynamics and the balance between formation and degradation of microbial byproducts. Soil microbes also indirectly influence C cycling by improving soil aggregation, which physically protects soil organic matter (SOM).

5.2.5. Microbial Exopolysaccharides

Presence of exopolysaccharides (EPS) surrounding microbial cells ensures to shield them against antimicrobial compounds and heavy metals. EPS can also retain water, thus protecting microbes and the environment against drought. In addition, other functions, such as adhesion, signaling with other microbes and plants, antioxidant, soil aggregation, carbon storage, and entrapment of nutrients have been also reported. Stable soil aggregates are important, long lasting source of carbon. In water stress condition exopolysaccharide play major role in the form of biofilm development, increasing soli aggregation and improving water holding capacity around plant root; and also improve the water stress tolerance ability of plant (Meena et al., 2017). EPS producing bacteria improve EPS also provide promising environment for maintaining moisture around root and rhizospheric area and protect the plant and bacteria against sear.

5.3.Strategies for abiotic stress strategies

Development of new microbe-based strategies and approaches can provide a powerful, sustainable option that could maintain the quality along with increased yield. Different strategies may be applied for enhancing the nutritional quality of food crops by using beneficial rhizospheric microorganisms: the manipulation of the crop microbiome *in situ*. Colonization signal molecules characterized from root exudates of leguminous plants are being added to the end product. The molecules being used in this refined technology include flavonoids, sugars, organic acids, amino acids, amines, and secondary metabolites that function as elicitor or signaling molecules for successful microbe-plant interaction (Skorupska et al. 2010). Commercial formulations containing biosignals are available for improvement in microbial colonization development of symbiotic relationship, triggering mechanisms to combat abiotic stress conditions, and induction of defense response in plants. Plant growth promoting rhizobacteria (PGPR) can potentially contribute significant role towards alleviation of abiotic stress in crop plants under present prospective of varying agro-climatic scenario; simultaneously the microbes can also help to reduce the excessive dependence on chemical fertilizers, thus maintaining the soil health. Increase the co-inoculation of stress tolerant microbial consortium of PGPM strains and mycorrhizal fungi in agriculture for enhancing plant growth under abiotic stress condition. Consortium of native bacterial strains is more advantageous over the individual strains originating from another niche. AMF and combined application of P solubilizers and N fixers are the best inoculants. The yield enhancement is more by the combinations of the two functional traits N fixation and P solubilization, than their distinct application as there is absence of competition and presence of positive interactions between the two traits (Schütz et al. 2018).

5.4.References:

Meena KK, Sorty AM, Bitla UM et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172. https://doi.org/10.3389/fpls.2017.00172

Skorupska A, Wielbo J, Kidaj D, Marek-Kozaczuk M (2010) Enhancing *Rhizobium* -legume symbiosis using signaling factors. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer, New York, pp 27–44

Cattelan AJ, Hartel PG, Fuhrman JJ (1999) Screening for plant growth promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Timmusk S, Abd El-Daim IA, Copolovici L et al (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:e96086

Meena KK, Sorty AM, Bitla UM et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172. https://doi.org/10.3389/fpls.2017.00172

Sorty AM, Meena KK, Choudhary K et al (2016) Effect of plant growth promoting bacteria associated with halophytic weed (*Psoralea corylifolia* L.) on germination and seedling growth of wheat under saline conditions. Appl Biochem Biotechnol 180:872–882. https://doi.org/10.1007/s12010-016-2139-z

Schütz L, Gattinger A, Meier M et al (2018) Improving Crop Yield and Nutrient Use Efficiency via Bio fertilization—A Global Meta-analysis. Front Plant Sci 8:2204. doi:10.3389/fpls.2017.02204

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21: 1–30.

Lone NA, Mir MR, Khan NA et al (2005) Effect of gibberellic acid on physiological attributes and yield of mustard (*Brassica juncea* L.). Appl Biol Res 7:24–26.

Gutierrez-Luna FM, Lopez-Bucio J, tamirano-Hernandez J et al (2010) Plant growth-promoting rhizobacteria modulate root-system architecture in *Arabidopsis thaliana* through volatile organic compound emission. Symbiosis 51:75–83. https://doi.org/10.1007/s13199-010-0066-2

Minerdi D, Bossi S, Maffei ME et al (2011) *Fusarium oxysporum* and its bacterial consortium promote lettuce growth and Expansin A5 gene expression through microbial volatile organic compound (MVOC) emission. FEMS Microbiol Ecol 76:342–351. https://doi. org/10.1111/j.1574-6941.2011.01051.x

Lemfack MC, Nickel J, Dunkel M et al (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42:D744–D748. https://doi.org/10.1093/nar/gkt1250

Supanekar S, Sorty A, Raut A (2013) Study of catechol siderophore froma newly isolated *Azotobacter* sp. SUP-III for its antimicrobial activity. J Microbiol Biotechnol Food Sci 3:270–273

Bianco C, Defez R (2009) Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. J Exp Bot. doi:10.1093/jxb/erp140

Chapter- 6

Nutritional management for abiotic stress management in livestock from drought prone areas.

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6.1. Introduction

Nutritional resource management is the important aspect of abiotic stress management in livestock in addition to genetic management. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The basic abiotic factors are soil, water, atmosphere, temperature and solar radiations/space (SWATS). The present classification of abiotic stress i.e. edaphic, drought, and atmospheric is based on the basic requirements of plants on these factors for their growth and production. Although edaphic factors have influence on whole biosphere, they do not directly influence animal growth and production. The nutritional requirements of animals are mostly fulfilled through plants which provide various required nutrients such as proteins, carbohydrates, fats, vitamins, minerals etc. However, nutrient requirements of animals are fulfilled through varied sources and not by any single type of plant alone. Hence, edaphic factors which directly influence plants have only indirect impact on animals. Besides climate and water, other abiotic factors that influence animals directly are very complex, depending on abiotic requirements for their sustenance, growth and production. The indirect effects of climate driven changes in animal performance result mainly from alterations in the nutritional environment (Valtorta 2010) besides direct effects of heat and water scarcity during drought cycles.

The inappropriate environmental factors influence directly as well as indirectly by altering supply of nonliving requirements to livestock in the specific production system. Drought is an extended period of water scarcity due to scarcity or the absence of rainfall, but related to inefficient water resource management. Drought is the frequent climatic condition adversely affecting agriculture/livestock production in drought prone areas of India. Drought is a natural hazard that differs from other hazards since it has a slow onset, evolves over months or even years, affects a large spatial extent, and cause little structural damage. Livestock plays important role in the economy of landless, marginal and small farmers (more than 80% of total farmers in India), besides sustaining farmers' income during scarcity/drought periods.

6.2.Drought Prone Areas in India

Out of the total geographical area of India, almost one-sixth area with 12% of the population is drought prone; the areas that receive an annual rainfall up to 600 mm are the most prone. The Irrigation Commission (1972) had identified 67 districts as drought prone. These comprise 326 talukas located in 8 states, covering an area of 49.73m ha. Subsequently, the National Commission on Agriculture (MOA 1976) identified a few more drought prone areas with slightly different criteria. Later, based on detailed studies, 74 districts of the country have been identified as drought prone. The districts having less than 60% of cultivated area under irrigation and possessing arid (31), semi-arid (133) and sub-humid (175) agro-ecosystems were identified and prioritized for Rainfed Area Development Programme (RADP). Recently extensive studies conducted under NICRA have led to identification of 100 districts in peninsular India highly prone to drought (Prasad et al., 2012). Adverse impact of drought is evident from the vast agricultural land left uncultivated and severe forage crisis for animals.

Broadly, the drought affected areas in India can be divided into two tracts. The first tract comprising the desert and the semi-arid regions covers an area of 0.6 million sq km. It is rectangle shaped area whose one side extends from Ahmadabad to Kanpur and the other from Kanpur to Jullundur. In this region, rainfall is less than 750 mm and at some places it is even less than 400 mm. The second tract comprises the dry region lying in the leeside of the Western Ghats up to a distance of about 300 km from coast. It is known as the rain shadow area of the Western Ghats; rainfall in this region is less than 750 mm and is highly erratic.

Outside these two main regions, there are isolated pockets which experience frequent droughts and are termed as drought prone areas. They are Coimbatore and Nellai Kottabomman districts in Tamil Nadu, Saurashtra and Kachchh regions, Janshi, Lalitpur region, Mirzapur plateau, Kalahandi region, Odisha, Purulia district of Paschim Bengal etc. (Sarkar 2011.)

6.3. Impact of Drought on Livestock

Besides direct impact of scarcity of water, drought also results in scarcity of nutritional resources and exposure of animals to adverse climatic conditions mainly heat stress. Scarcity of forages during drought can increase the risks of animal poisonings and nutritional imbalances. The impaired water quality, feed quality, nutritional deficiency and increased incidence of plant poisonings are some drought-related threats to cattle health and productivity. (Poppenga and Puschner). Due to reduced availability of fodder, animals are forced to consume other vegetations or non-conventional feed stuffs with increased risk of exposure to anti-nutritional factors. In addition, once the drought-breaking rains occur, the grazing conditions for cattle may dramatically change and pose additional health risks. The conditions mostly commonly associated with the end of severe drought conditions include bloat, certain deficiency problems, plant poisonings, and clostridial diseases. The other impacts of drought are changes in production systems which include migration of livestock farmers to surplus areas, sale of animals for slaughter and shifting from large ruminant based systems to small ruminant systems.

6.4. Drought Stress Management through Nutritional Technologies

There are many challenges for sustaining livestock wealth due to recurrent drought or delayed monsoon like situations in drought prone areas in the country. Research efforts regarding suitability of fodder species for increasing production, alternate fodder sources and optimization for their use, storage and transport of fodder, optimizing nutrient availability and utilization by the different livestock species in target areas are warranted for sustainable livestock production in drought prone areas.

6.4.1. Development and use of drought tolerant fodder varieties

Research efforts should be for increasing the fodder yield of cultivated fodder crops on agricultural lands as well as on wastelands and community pastures (Hegde, 2010). The strategy should include selection and breeding of high yielding and stress tolerant as well as short duration fodder crops and varieties. Importance may be given to improve the yields through sustainable production practices, efficient conservation practices and strengthening the value chain of dairy and meat producers by providing various critical services required to improve productivity and sustain livelihood. For this joint efforts of various government and non- government agencies are important. A comprehensive review of the improved fodder crop varieties released/notified during the past three decades, fodder production systems and packages of practices for important fodder crop, intensive forage sequences recommended for different regions has been provided in Handbook of agriculture, (2010).

6.4.2. Hydroponics

There is renewed interest in hydroponic fodder as a feedstuff for sheep, goats, and other livestock. The yield and quality of sprouts produced is influenced by many factors such as soaking time, grain quality, grain variety and treatments, temperature, humidity, nutrient supply, depth and density of grain in troughs and the incidence of mould (Sneath and McIntosh 2003). The technology of hydroponic systems is changing rapidly with systems today producing yields never before realized. The future for hydroponics appears more positive today than any time over the last 50 years. Methods and technologies that can contribute to improved water use efficiency and productivity merit closer consideration like hydroponic technique (Al-Karaki and Al-Hashimi 2012). Hydroponically produced fodder was found to enhance the efficiency of water use. Bradley and Marulanda (2000) reported that hydroponic green fodder production technique requires only about 10–20% of the water needed to produce the same amount of crop in soil culture. More research efforts regarding water saving options including use of treated waste water for hydroponic green fodder production need to be carried out for applying in drought prone areas of India.

6.4.2. Hay and Silage making

Hay making and ensiling are the only options available to farmers wanting to conserve forage on a large scale. In drier climates, haymaking is still important. However, there has been a trend over the last 30 years or so for the proportion of forage conserved as silage to increase, while the proportion dedicated to hay has declined (Wilkinson et al. 1996). Ensiling offers many advantages over haymaking. Large quantities of forage can be conserved in a short time, forage conservation is less weather dependent and thirdly, silage is well suited to mechanization. However, a major disadvantage associated with silage making is that the feeding value of the resultant forage is reduced relative to that of the original crop (Charmley, 2000). Silage is made of forages, crop residues, or agricultural and industrial by-products that have been preserved by natural or artificial acidification, for use as animal feed in periods when feed supply is inadequate (Mannetje, 1999).

According to Charmley, 2000, the possibility that in future, silages will have superior feeding value to the original crop is realistic. Physical treatments can break down barriers to improve intake and digestibility. Predictable silage fermentation can be used to optimize rumen function. More research efforts, besides popularization of technique, to improve silage intake and utilization using locally available forages are required to overcome the scarcity of fodder during drought cycles in different regions of India.

Making silage from drought damaged crops, need to be assessed in drought prone areas in India as a nutrition management option. Availability of sugarcane tops in the drought prone areas need to be exploited as effective drought stress management option. During drought conditions plant growth is impaired and nitrates can accumulate in the plant. Nitrates are normally taken up by plants from the soil and utilized for the synthesis of plant protein. Elevated nitrate levels can also occur in summer annual forages subjected to drought stress. Weeds commonly found in corn fields such as pigweed, ragweed, lambsquarter, nightshade, and Johnsongrass can also accumulate toxic levels of nitrates, under drought conditions. High nitrates concentrations in corn plants and corn silage can potentially be toxic to cattle (Wright 2015).

6.4.3. Complete feed blocks

The crop residues have low nutritional value and are bulky and fibrous. In addition, availability of crop residues varies with season and region. In some regions there is deficiency of crop residues, while in some other regions they are available in abundance but are largely wasted. Under emergency situations complete feed technology has been used to save the animals from hunger and death. Based on the productivity levels of animals, the Densified Total Mixed Ration Blocks (DTMRBs) or the densified total mixed ration pellets (DTMRPs) of different formulations can be made using different ingredients, including minerals, vitamins and feed additives. Thus, the technology of straw-based densified complete feed as blocks or pellets could play an important role in providing balanced rations to livestock in the tropical regions of green forage scarcity. The technology offers a means to increase milk and meat production in the tropics apart from having other advantages such as: decrease in environmental pollutants (including methane emission), increase in income of farmers, decrease in labour requirement and time for feeding and reduction in transportation cost of straw. The technology also has the potential to alleviate regional disparity in feed availability, as the block or pellet making units can be set up to act as 'Feed Banks' in regions of abundant crop residue availability. It could also provide complete feed to livestock under emergency situations created by natural calamities such as drought and man-made conflicts (Walli et al., 2012). However, there is a need to take up further research on energy cost of straw transportation and feed densification and how to reduce it. Research may also be taken up for monitoring the quality of the processed feed to check that the nutrients are not diluted by the addition of more of non-nutritional feed additives. Feeding of complete ration in mash form is beneficial in terms of feed intake, body weight gain, nutrient utilization and feed conversion efficiency in growing crossbred female calves in comparison to conventional feeding system and also with complete feed in block form (Sharma et al., 2010).

6.4.4. Urea molasses treatment

It is generally recommended to avoid urea treatment during periods of drought. However, urea treatment of poor quality fodder if done judiciously under controlled condition is beneficial to sustain the periods of drought.

6.5. Nutrient management during drought stress

During periods of scarcity nutrient management of individual animals is important to sustain health and production or even save the life of animals. Feeding of concentrate mixtures and Mineral/vitamin supplementation based on the requirement may be provided under the guidance of experts.

6.6. Novel feed resources/ Alternate feed

Search for alternate feed resources and research for its judicious use need to be carried out for the different agro-ecological systems. Several newer feed resources have been evaluated and found useful for feeding. Incriminating factors have been identified in unconventional feeds and methods for their detoxification have been evolved. Protein cakes after oil extraction from seeds of neem, castor, karanj, palm and mahua have been evaluated and found suitable after detoxification to use for feeding. However, largely this technology is not yet adopted by end users.

6.7. Drought Stress Management through Management of Nutritional Resources:

During periods of drought cycles there is overall shortage of feeds and fodder for livestock in the area. Unavailability of forages results in shortage to provide the needed dry matter intake and subsequently overall nutrients the animal needs. In these situations it becomes necessary to provide a supplemental forage source to meet this need. In many cases, these forages are substandard so additional supplementation may be needed as well to maintain a base-line production level. Providing inadequate levels of protein and energy always reduces performance in some manner and is stressful to the animal.

6.8. Deficiency estimates of forages

The data/estimates of fodder production in the country vary widely. Fodder production and its utilization depend on the cropping pattern, climate, socioeconomic conditions and type of livestock. At present, the country faces a net deficit of 61.1% green fodder, 21.9% dry crop residues and 64% feeds (Anonymous, 2010).

Although there are variable estimates of feed and fodder availability in the country, all of the estimates point towards overall deficient status of feed and fodders for livestock even in absence of drought. The projected deficit of green and dry fodder appears to be agrevated during near future.

Table: Status of feed and fodder (DM basis) in India

Feeds	**Available (MT)**	**Required (MT)**	**Deficit (%)**
Dry fodder	**365**	**412**	**11**
Concentrate	**34**	**47**	**28**
Green Fodder	**126**	**193**	**35**
Total	**526**	**652**	**19**

NIANP 2005

6.9. Strategies for management

During drought cycles these situations of deficit forages are further aggravated in drought prone areas with severe negative impact on livestock population. To address these problems following strategies are suggested by National disaster management authority (NDMA).

Strategies suggested by NDMA	Interventions needed
i. Assessment of need for fodder will be done well in advance. If a deficit is identified, ways and means to fill the gap will be explored including supplies from the nearest area, within the mandal, within the District, or in the nearby State.	Timely and realistic assessments and ensuring availability of fodder. Exploring alternative sources.
ii. Raising of fodder in Government as well as farmers' lands with buy back arrangements for fodder cultivated will be promoted.	Initiatives of government agencies with area specific programmes required.
iii. Use of tank bunds for fodder cultivation.	Suitable guidelines with area specific varieties needed.
iv. Utilizing the period between crops for fodder cultivation.	Suitable guidelines with area specific varieties needed.
v. Distribution of fodder produced within a State in nearby areas.	Initiatives of government and non-government agencies
vi. Establishment of fodder banks.	
vii. Conserving fish and aqua culture during droughts.	Need to be coupled with water conservation practices
viii. Utilizing the assistance of Ministry of Railways in transport of fodder and drinking water from unaffected areas to those affected.	Initiatives of government agencies required.
ix. Organizing online availability of information relating to demand and supply of fodder	
x. Undertaking market intervention to keep the prices reasonable.	
xi. Intensification of water conservation measures in the villages.	

6.10. Knowledge of livestock farmers about Deficiency or excesses of nutrients

Livestock farmers feed their animals based on their traditional knowledge and availability of forages. In general there is lack of scientific/balanced feeding of livestock. Imbalanced feeding leads to excess feeding of some nutrients whilst others remain deficient. This not only reduces milk production and increases costs per kg milk, but also affects various physiological functions including long term animal health, fertility and productivity. The farmers should be trained about balanced feeding of livestock and using nutritional technologies such as hay/silage making, hydroponics, and feed block with TMR etc. during routine management

Detailed knowledge of the nutritional requirements of animal species and during different stages of growth and status of production is required.

6.14. Conclusions

As most of the abiotic stressors affect livestock through their diet and impacts of most of stressors may be managed by providing appropriate nutrients, nutrition and management of nutritional resource. Nutritional management is important aspect of abiotic stress management in livestock to reduce impact of climate change issues particularly repeated or extended periods of drought. Defining the availability of various nutritional resources, their nutritional composition, judicious use and strategic preservation through silage making, use of hydroponics and TMR blocks is required to avoid stress in productive animals and sustain the livestock population in drought prone areas.

6.15. Reference

1. Anonymus 2010. National Disaster Management Guidelines: Management of Drought. A publication of the National Disaster Management Authority, Government of India. ISBN 978-93-80440-08-8, September 2010, New Delhi.
2. Bradley P.and Marulanda C., , 2000. "Simplified hydroponics to reduce global hunger," Acta Horticulture, vol. 554, pp. 289–295.
3. Valtorta S.E. (2010) Animal production in a changing climate: impacts and mitigation http://animalsciences.missouri.edu/research/bec/Brody%20Lecture%20-%20Valtora.pd
4. Prasad, Y.G., Venkateswarlu,B., Ravindra Chary, G., Srinivasarao, Ch., Rao, K.V., Ramana, D.B.V., Rao, V.U.M., Subba Reddy, G. and Singh, A.K. (2012). Contingency Crop Planning for 100 Districts in Peninsular India. Central Research Institute for Dryland Agriculture, Hyderabad 500 059, India. p. 302

5. FAO. 2012. Crop residue based densified total mixed ration – A user-friendly approach to utilise food crop by-products for ruminant production, by T.K. Walli, M.R. Garg & Harinder P.S. Makkar. FAO Animal Production and Health Paper No. 172. Rome, Italy.
6. D'Mello, J.P.F. 1995. Antinutritional substances in legume seeds. In J.P.F. D'Mello and C. Devendra, eds. Tropical legumes in animal nutrition. Wallingford, UK, CAB International. p. 135-172.
7. FAO. 2012. Balanced feeding for improving livestock productivity – Increase in milk production and nutrient use efficiency and decrease in methane emission, by M.R. Garg. FAO Animal Production and Health Paper No. 173. Rome, Italy.
8. Frutos P., Hervás G., Giráldez F. J. and Mantecón A. R. 2004. Review. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research 2 (2), 191-202
9. Hammerschmidt R. 1999. PHYTOALEXINS:What HaveWe Learned After 60 Years? Annu. Rev. Phytopathol. 1999. 37:285–306.
10. Hinton M. H. 2000. Infections and intoxications associated with animal feed and forage which may present a hazard to human health. The Veterinary Journal, 159, 124–138.
11. Katole S. B., Puneet Kumar[1] and Patil R. D. 2013. Environmental pollutants and livestock health: a review. Veterinary Research International,Vol. 1(1) : 1-13

Chapter- 7

Farm Pond based Aquaculture Model: Resource use

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7.1. Prologue

The majority of the Indian population is dependent on agriculture and allied sectors for their livelihood and income. Therefore, maximum emphasis is given to the development and improvement of these sectors. However, limited irrigation is one of the important and critical constraints in these sectors as only 35% of the net sown area in India is under complete irrigation. To overcome irrigation problems as well as to boost the agriculture production the government encouraging construction of farm ponds which can harvest of rainwater and subsequently storing this water for later (during drought) use of agriculture. These farm ponds which are constructed under different agricultural scheme in the farmers land has scope to utilize for aquaculture without affecting the water level in farm pond. The farm pond based aquaculture is potential option to improve farmer's surplus income and ensure their sustainable livelihood. Fish culture in such water bodies has potential to increase fish production and economic return per unit area of land. This model of producing fish fingerlings results in increasing the fingerling production and will take 'Mission Fingerling' programmes as forward linkage in Blue revolution scheme. It is expected that this additional water area will prove a boon to 'Mission Fingerling' programmes to achieve blue revolution.

7.2. Introduction

Past strategy for development of agriculture sector in India has focused primarily on rising agricultural output and improving food security. It is obvious that if inflation in agriculture price is high, farmer's income in nominal terms will double in much shorter period. This implies that the ongoing and previously achieved rate of growth in farmer income has to be accelerated. Therefore realise the need to pay special attention to the farmer, the Ministry of Agriculture and Farmers Welfare set the goal of doubling farmers income by the year of 2022 and took strong measure for the same to harness all possible sources of growth in farmers income within as well as outside agriculture sector. MoA mentioned the major sources of growth operating within agricultural sector are:

1) Improving in productivity
2) Resource use efficiency or saving in cost of production
3) Increase in cropping intensity
4) Diversification towards high value crop
5) Shifting cultivator from farm to non-farm occupation
6) Improvement in terms of trade for farmers or real price

So, keeping in the view of above major possible sources of growth of income, the farm pond based aquaculture, based on two above sources i.e. 1) Resource use efficiency or saving in cost of production and 2) Diversification towards high value crop.

7.3. Farm ponds a boon for farmers

Since Climate change impacts agriculture by reducing yield due to intensity of drought, rise in temperature, variability in precipitation and reducing the availability of several natural resources, water being the most prominently commodity. To overcome this Agriculture Department of the Government of India has initiated a '*National Horticulture Mission'*, *Maharashtra Rural Employment Guarantee Scheme*, *Rashtriya Krishi Vikas Yojana* (RKVY). Maharashtra State initiated significant efforts in this direction and a scheme popularly known as *"Magel Tyala Shet Tale*" (Farm pond on Demands) and *Jalyukt Shivar* (JYS). In Andhra Pradesh, mandal committee will prepare a plan on '*Neeru-Chettu'*, NREGA and farm ponds construction. Under '*Panta-Kunta'* programme, two to five small farm ponds will be dug in every village. All schemes offers a subsidy for digging and lining the farm ponds which to ensure water storage for longer period. Farm ponds are manmade tanks constructed for holding water which could be used during scarce season to ensure lifesaving irrigation for the crops. Very Few studies conducted on aquaculture of farm pond provides information on the aquaculture.

7.4. Smallholder fish production systems

Todays, the majority of the individual farmers have farm pond and has scope to utilize for aquaculture without affecting the water level for future use. At the same time scarcity of water is a major problem faced by the farmers and a slew of measures to protect remuneration, keep input costs low and open up alternative forms of income will help the farmers getting surplus income. So, Fish culture in farm pond has potential to increase fish production system and economic return per unit area of land. The investment costs are small and a return on the investment is possible in as little as three months which is totally surplus income of the farmers in existing resources. It appears to be a highly suitable livelihoods option for people who have farm pond with limited capital investment and operating costs of around Rs.7650 for a 30 X 30X 3 m farm pond, generating ₹ 28000-30000 of revenue.

Now days, major concern in freshwater aquaculture is to increase production per unit area by applying different innovations and technologies in existing water resources. The production from marine sector has almost reached its potential as most of the resources have already been overexploited and therefore there is little scope for increasing the production from that source. On the contrary, most of the inland fishery resources are underexploited and there is tremendous scope for triggering fish production from different inland fisheries resources. A study made by the International Water Management Institute (IWMI) revels that by 2025 nearly 1/3rd of world population would live in the regions of severe water scarcity and the same proportion of the population in India could face absolute water scarcity. Therefore a major focus will be on the judicious management of water resources like farm pond and do develop strategies for its efficient and multiple uses.

These water bodies' uses for agriculture use during the drought period and it can be parallel used for aquaculture by stocking fish species such as *Labeo rohita, Catla catla* and *Cirrihinus mrigala*, GIF Tilapia *Oreochromis niloticus*, *Pangasianodon hypophthalmus*. This model of producing fish fingerlings results in increasing the farmer's income. It is also expected that this additional water area will prove a boon to 'Mission Fingerling' programmes to achieve blue revolution. In the light of this perspective, Farm Pond based Aquaculture Model could take a viable option for doubling farmer income in India.

7.5. Boosting fingerling supply

A key constraint to aquaculture in large water bodies is the supply of fingerlings especially those of a sufficiently large size to grow quickly to market size, in seasonal ponds or to avoid predation in perennial ponds. The DARE realised the fish fingerling production is the single most important critical mission visualised to achieve fish production targets under the Blue Revolution, The *Neel Kranti* Mission. The mission seeks to enhance fisheries production to 15mmt from 10.79 mmt by the end of the year 2020-21, under the Blue Revolution. Large-scale implementation of farm pond excavation scheme is going to be a major boon for the fingerling production. There was no systematic supply chain for fish fingerling in the India, where it can make backward linkages for fish growers. Farm pond based aquaculture makes bridge between fish hatchery to fish grower.

7.6. Farming strategies

Nursing fish seed is arguably the simplest component of farm pond based aquaculture and a suitable entry-point into aquaculture for farmers and fishers. Between hatchery operations, which produce spawn, and on-growing operations, which raise carp to market size, is a short but vital stage where spawn are nursed in farm pond to fingerlings – the size at which they can be stocked in larger seasonal or perennial ponds. The nursery rearing involve nurturing of 3 days old spawn which has just begin to eat and continue for the period of 3 months to raise the fingerling size. The different management strategy involved to fingerling production is:

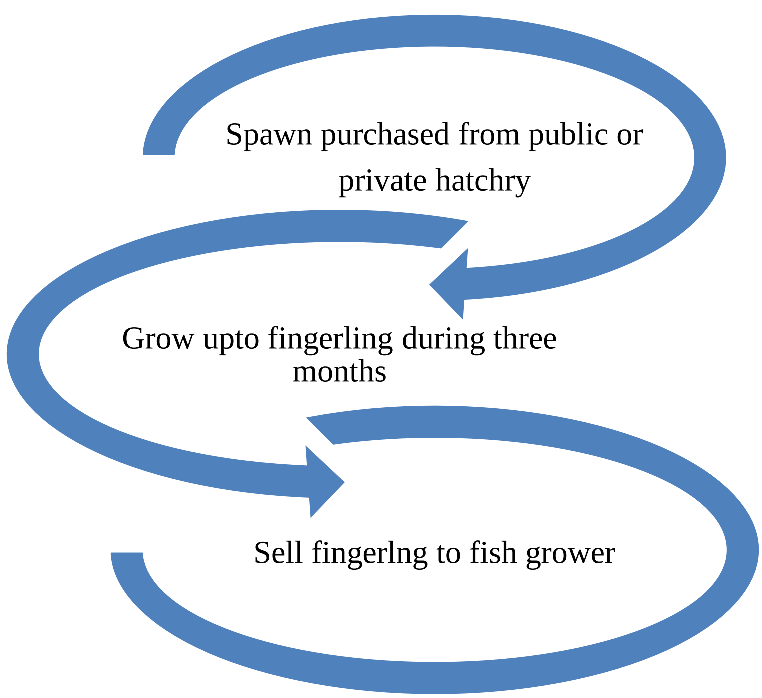

Fig.1 Backword and farwrd linkage of farmers

7.6.1. Nursery farming and management in Farm pond

In farm pond based aquaculture model, emphasis is given on boosting the primary productivity by manure and fertilization. This will ensure enough availability of plankton as natural food. In addition, supplementary feed plays an important role and fed @ of 5-8 % of body weight. Broadly, the various steps involved in the management of farm ponds may be classified as (i) Pre-Stocking Management, (ii) Stocking Management (iii) Post-Stocking Management operations

7.6.2. Pre-stocking management

Pre-stocking management aims at proper preparation of pond to remove the causes of poor survival, unsatisfactory growth, etc. and also to ensure ready availability of natural food in sufficient quantity and quality for spawn/fry/fingerlings to be stocked. The first step in pre-stocking management is to ploughing (without lining farm pond), eradicate aquatic vegetation and predatory & weed fishes. The pond is fertilized with organic manures and inorganic fertilizes, the cow dung is applied generally @ of 1000 kg/ha, urea @ 100 kg/ha/year and single supar phopsphte @ 50 kg/ha/year. The oil emulsion dose (diesel and detergent @ 0.5 litre & detergent 200 gm) should be applied prior to 2-3 days of stocking. Pre-stocking part of the management involves the following sequential measures (Fig. 1-6).

Fig. 1 Pond bottom drying

Fig. 2 Ploughing

Fig. 3 Liming

Fig. 4 Manuring

Fig. 5 Netting for irradiation of insect

Fig. 6 *Hapa* installation

Stocking management:-

This may be conveniently done under fixed conditions by fixing a '*Hapa*' in a pond and releasing spawn (6 mm) in it. Comfortable behaviour of spawn for about 24 hours confirms complete detoxification and the pond should be regarded as ready for stocking (See fig. no 7-8).

Fig. 7 conditioning of spawn

Fig. 8 Acclimatization of seed

Nursery ponds are stock with self-produced or procured 3 – 4 old spawn usually in the morning hour. Acclimatization is an important aspect for spawn survival and needs attention to avoid any abrupt change in a water quality, importantly temperature & pH. The rate of stocking in a well prepared farm pond with adequate fish food organisms can be as high as 1.5-2 lakh spawn/ farm pond (30 x 30 x 3 m farm pond). However, the survival level decreases with the increase in stocking density.

Table 1. Rates of stocking density and its biomass in farm pond

Pond size in M	Area in m^2	Spawn stocking	Biomass (gm) (Avg wt 0.0014 gm)
15X15	225	50000	70
20x20	400	100000	140
30X30	900	200000	280

7.6.3. Post-stocking management

Post-stocking management involves maintenance of pond environment are harnessing the pond productivity in the form of natural fish food and maintain supplementary feeding and health care.

7.6.4. Supplementary feeding

Soon after stocking, the fish start grazing natural food available in the pond irrespective of their stage of life cycle. Spawn feeds voraciously on plankton. Therefore, immediate steps must be taken for providing supplementary feed. In the case of nursery ponds where spawn are reared for about a fortnight up to fry stage, the form in which the supplementary feed is given is also important. In the nursery ponds the feed should be provided in finely powdered form and may be broadcast over the pond surface. In the case of rearing, stocking and brood stock ponds, the supplementary feed mixture should be mixed with enough water to make dough and applied into feeding trays fixed in the ponds. Better results can be obtained if the feed mixture is pelletized and fed to fish.

Table 2. Rates of Daily Supplementary Feeding management of farm pond

Period (Day from the date of stocking)	Rate of feeding	Amount of feed for 1 lakh spawn
1 -5	2 times the total initial weight	280 g/day
6 - 15	4-6 times the total initial weight	560-840 g/day
16-30	8-10 times the total initial weight	1120-1400 g/day
30-90	50-80 % the total initial weight	-

7.6.5. Health management

Under, feeding leads to malnutrition, resulting in growth retardation and low disease resistance. Liver lipoid disease, scoliosis and lordosis *etc*. are the examples of such mal-nutritional disorders. Many of the fish may carry small numbers of pathogens like bacteria, virus, fungi and parasites, either at chronic low-grade infections or serving as carriers. The best way to avoid disease outbreak in the pond during culture is through taking preventive measures which are ensured by proper management of the soil and water quality, following proper feeding schedule, use of balanced feed, periodic sampling for health check, etc.

7.7. Economics of Fingerling production in Farm pond (30 X 30 X 3 M)

After due rearing phase it is proposed to harvest and sell live Fingerlings by way of oxygen packing to needy fish farmers and fishermen.

Table 3. Economics of Fingerling production in Farm pond during three months

Sr. no	Particulars	Unit	Rate (₹/Unit)	Quantity (Unit)	Amount (₹)
1	Spawn of catla, Rohu & Mrigal	Lakh	1500	2.0	3000
2	Cow dung	KG	0.5	1000	500
3	Inorganic fertilizer (Urea & SSP)	KG	0.5	10	50
4	Supplementary feed	KG	60	35	2100
5	Miscellaneous	-	-	-	2000

6	Total Expenditures			7650	
7	Yield (Fingerling)	No.	0.6	60000	36000
8	**Net profit (7-6)**			₹ **28350**	

Keeping in the view the local climatic condition with shorter growing period, the better management practice is introduces in farm pond based aquaculture system. The local availability of carp seed strengthen the private fish farming and adopting this management the farmers will generate ₹ 28000-30000 of revenue from 30 X 30 X 3 m farm pond every year.

Chapter- 8

Livestock and climate change

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8.1. Introduction

The world population has increased by 1 billion over last 12 years reaching 7.6 billion in 2017 and is expected to be 9.8 billion in 2050. With the increase in population and global standard of living, demand for agricultural products also will increase by about 70%. However, total global cultivated land area has not changed since 1991, reflecting need for increased productivity and intensification efforts. Livestock products are an important agricultural commodity for global food security because they provide 17% of global kilocalorie intake and 33% of global protein consumption. Population growth, urbanization, and income rise in developing countries are the main driver of the increased demand for livestock products. The issue of 'Climate change and livestock' has two facets, the impacts of climate change on livestock production along with food security and the livestock sector's impact on climate change.

8.2. Impact of climate change on livestock

Intergovernmental Panel on Climate Change's report identified increase in global average surface temperature between 0.3 °C and 4.8 °C by 2100. The potential impacts on livestock include changes in production and quality of feed crop and forage, water availability, animal growth and milk production, diseases, reproduction, and biodiversity. The impacts of climate change on livestock production factors are presented in Fig. 1.

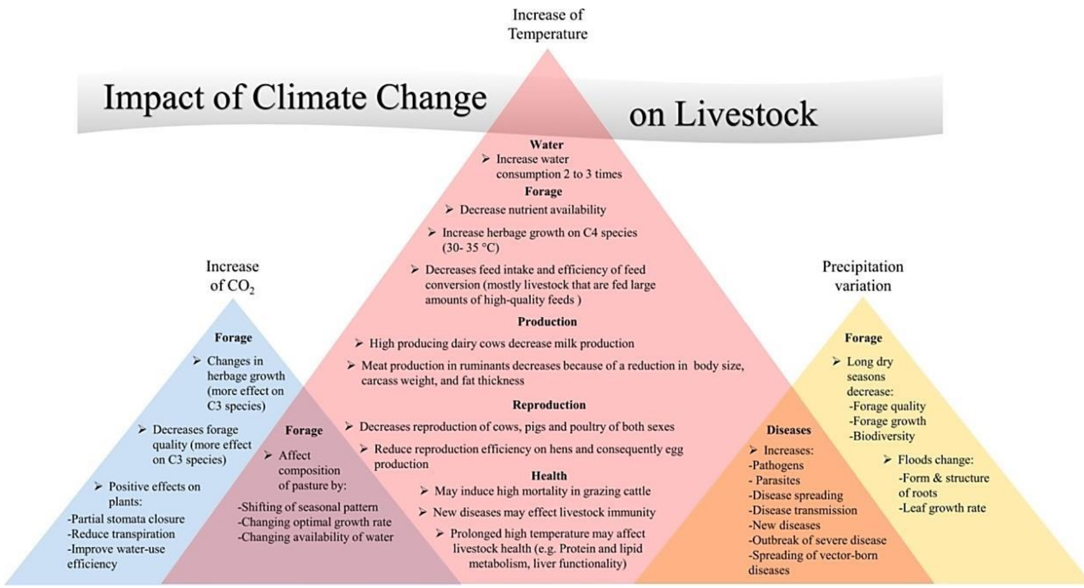


Fig. 1: Summary of impacts of climate change on Livestock.

8.2.1. Quantity and quality of feeds:

Quantity and quality of feed will be affected mainly due to an increase in atmospheric CO_2 levels and temperature and are dependent on location, livestock system, and species. An increase of 2 °C will produce negative impacts on pasture and livestock production in arid and semiarid regions and positive impacts in humid temperate regions. The length of rainy season is also an important factor for forage quality and quantity because it determines the duration and periods of available forage.

8.2.2. Water scarcity

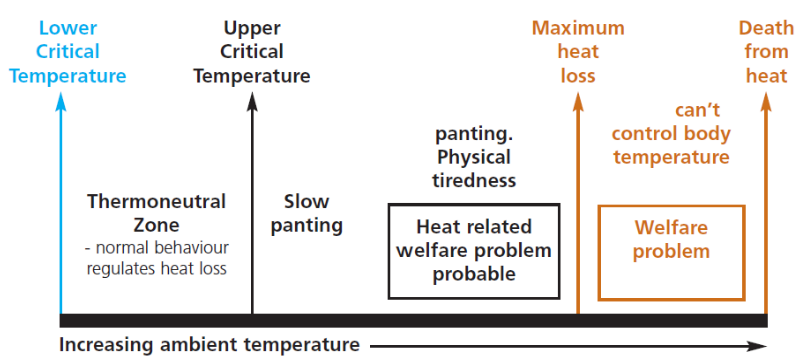
Global agriculture uses 70% of fresh water resources, making it the world's largest consumer. Water availability issues will influence the livestock sector, which uses water for animal drinking, feed crops, and product processes. The livestock sector accounts for about 8% of global human water use and an increase in temperature may increase animal water consumption by a factor of two to three. However, global water demand is moving towards increased competition due to water scarcity and depletion, where 64% of the world's population may live under water-stressful conditions by 2025.

8.2.3. Livestock diseases

The effects of climate change on livestock diseases depend on the geographical region, land use type, disease characteristics, and animal susceptibility. Animal health can be affected directly or indirectly by climate change, especially rising temperatures. The direct effects are related to the increase of temperature, which increases the potential for morbidity and death. The indirect effects are related to the impacts of climate change on microbial communities (pathogens or parasites), spreading of vector-borne diseases, food-borne diseases, host resistance, and feed and water scarcity. Climate change may induce shifts in disease spreading, outbreaks of severe disease, or even introduce new diseases, which may affect livestock that are not usually exposed to these types of diseases.

8.2.4. Heat stress

All animals have a thermal comfort/thermo-neutral zone, which is a range of ambient environmental temperatures that are beneficial to physiological functions. When temperature increases more than the upper critical temperature, the animals begin to suffer heat stress (Fig. 2). Heat stress on livestock is dependent on temperature, humidity, species, genetic potential, life stage, and nutritional status. Animals have developed a phenotypic response to heat stress called acclimation. Acclimation results in reduced feed intake, increased water intake, and altered physiological functions such as reproductive and productive efficiency and a change in respiration rate. Heat stress decreases forage intake, milk production, the efficiency of feed conversion, and performance. Warm and humid conditions cause heat stress, which affects behaviour and metabolic variations on livestock or even mortality.



Heat stroke

Fig. 2: Thermal comfort/thermo-neutral zone in livestock.

8.2.5. Biodiversity

Biodiversity refers to a variety of genes, organisms and ecosystems found within a specific environment and contribute to human well-being. IPCC Report states increase of 2 to 3 °C may result in 20 to 30% of biodiversity loss of plants and animals (Fig. 3). By 2000, 16% of livestock breeds (water buffalo, cattle, goat, pig, sheep, and horse) were lost. The biodiversity loss is mainly because of the practices used in livestock production that emphasize yield and economic returns and marginalization of traditional production systems where other considerations are also important (ability to withstand extremes). Livestock will be highly affected by climate change and biodiversity loss.

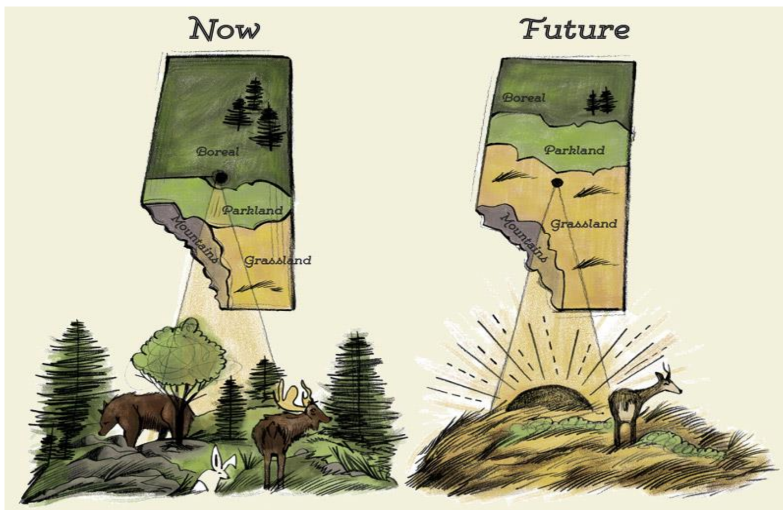


Fig. 3: Loss of biodiversity due to climate change.

8.2.6. Food security

Livestock contributes greatly to food security as they are suppliers of global calories, proteins, and essential micronutrients; are produced in areas with difficulty growing crops; utilizing feed not appropriate for human consumption; providing manure for crop production. Livestock production may decrease through declining forage quality and quantity and/or by reducing animal feed intake. Under stress, animals will use the available nutrients to first maintain their physiological needs, then for growth or milk production, and finally for reproduction. Climate change also affects nutritional content of livestock products because of potential increases in pathogens and diseases in their food and effects on the animals themselves.

8.3. Impact of livestock on climate change

Livestock influence climate through land use change, feed production, animal production, manure, and processing and transport (Fig. 4). The livestock sector is often associated with negative environmental impacts such as land degradation, air and water pollution, and biodiversity destruction. Increases in livestock production are expected to originate from a declining natural resource base, which will cause further environmental damage without proper natural resources management.

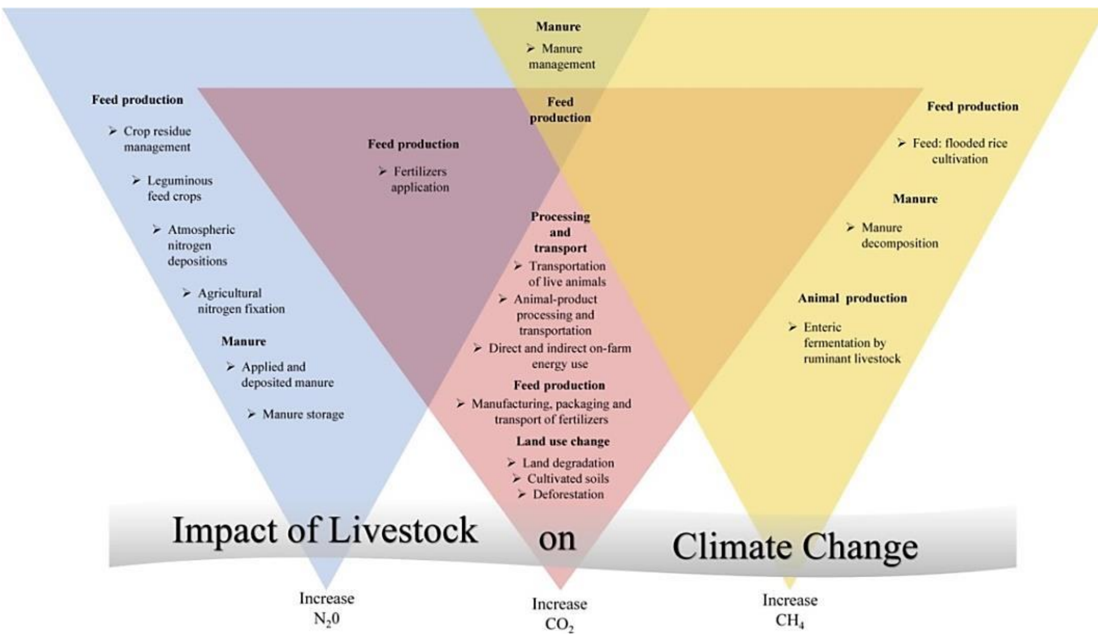


Fig. 4: Summary of impacts of livestock on climate change.

8.3.1. GHG emissions

Livestock contribute 14.5% of the total annual anthropogenic greenhouse gases (GHG) emissions globally. The primary livestock GHG emissions are CO_2, CH_4, and N_2O. Globally livestock contribute 44% of anthropogenic CH_4, 53% of anthropogenic N_2O and 5% of anthropogenic CO_2 emissions (Fig. 5).

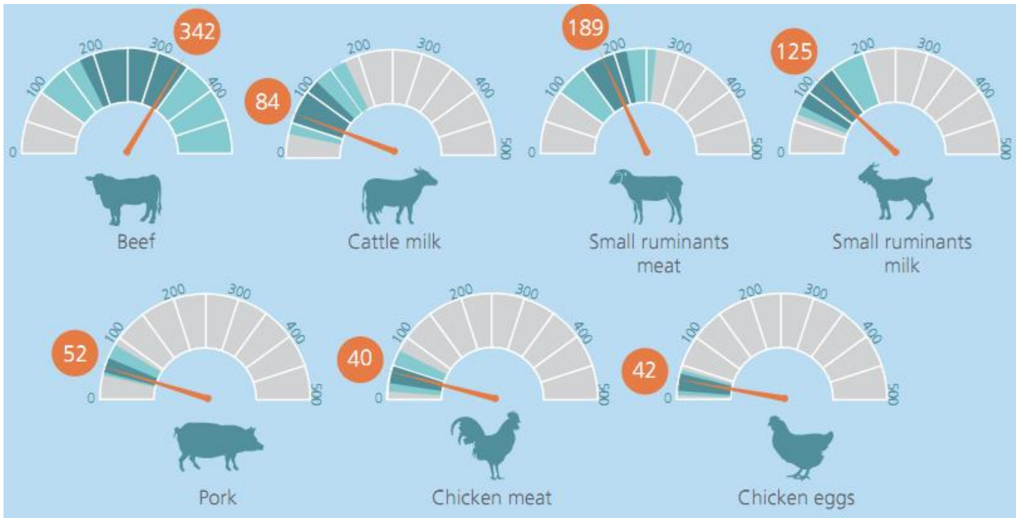


Fig. 5: Livestock GHG emissions.

Higher concentrations of these gases can be explained by lower efficiency and productivity of livestock system due to excess loss of nutrients, energy, and organic matter. Enteric fermentation is the largest contributor of the sector's emissions with 39.1%. However, contribution to GHG emissions varies depending on the type of farming system and region (Fig. 6).

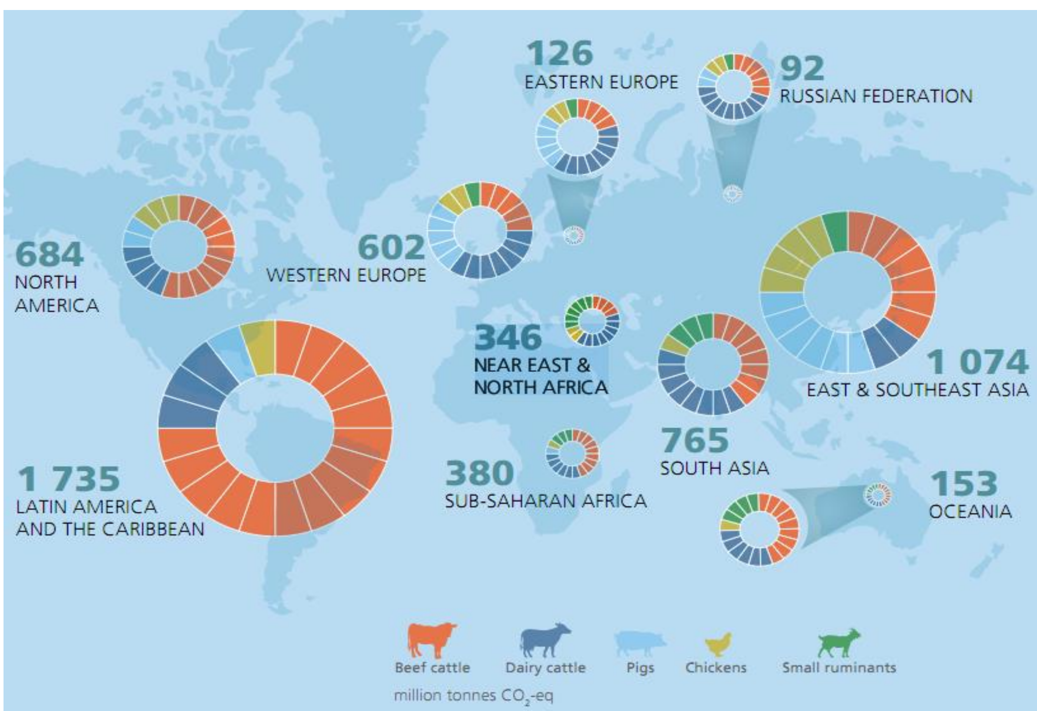


Fig. 6: Regional distribution of emissions from livestock

8.3.2. Land use

The increasing demand for livestock products has significantly changed the natural landscape. Deforestation, cultivated soils, and land degradation due to livestock grazing are the main source of CO_2 emissions. From total livestock GHG emissions, 9.2% is attributed to land use change, where 6% is due to pasture expansion and 3.2% is due to feed crop expansion. Due to climate and soil characteristics, the expansion of pasture into marginal areas is limited; therefore, they could only expand into areas with agro-ecological potential. Grazing management that can increase carbon sequestration are; maintaining effective stocking rate in pastureland; rotational grazing and excluding degraded pasturelands from livestock grazing.

8.3.3. Fossil fuel use in livestock production

On-farm fossil fuel use in livestock production produces 50% more CO_2 emissions than manufacturing N fertilizers for feed. The livestock sector includes direct and indirect on-farm fossil fuel use for machinery operations, irrigation, heating, cooling, ventilation, production of herbicides and pesticides, etc. More than half of fossil-fuel use is attributed to feed production. By assuming CO_2 emissions from on-farm fossil fuel use are double that of manufacturing N fertilizers, and adding emissions related to livestock rearing, on-farm fossil fuels account for 90 million tonnes of CO_2 per year.

8.3.4. Livestock production

In general, livestock respiration is not counted as a net source of CO_2 emissions as they are part of the global biological system cycle. The vegetation consumed by the animal originates from the conversion of atmospheric CO_2 to organic compounds or biomass; hence, it is assumed that the consumed amounts of CO_2 in vegetative form are equivalent to those emitted by the livestock. Conversely, the animal is a carbon sink because a fraction of the carbon consumed is absorbed in the live tissue of the animal and products such as milk. If GHG emissions are estimated based on commodities, beef cattle contribute the most with 41% of the sector's emission, followed by dairy cattle (20%), swine (9%), buffalo (8%), poultry (8%), and small ruminant (6%). Enteric fermentation is the largest source of GHG emissions from cattle, buffalo, and small ruminants, comprising between 43% and 63% of the livestock sector emissions (Fig. 7).

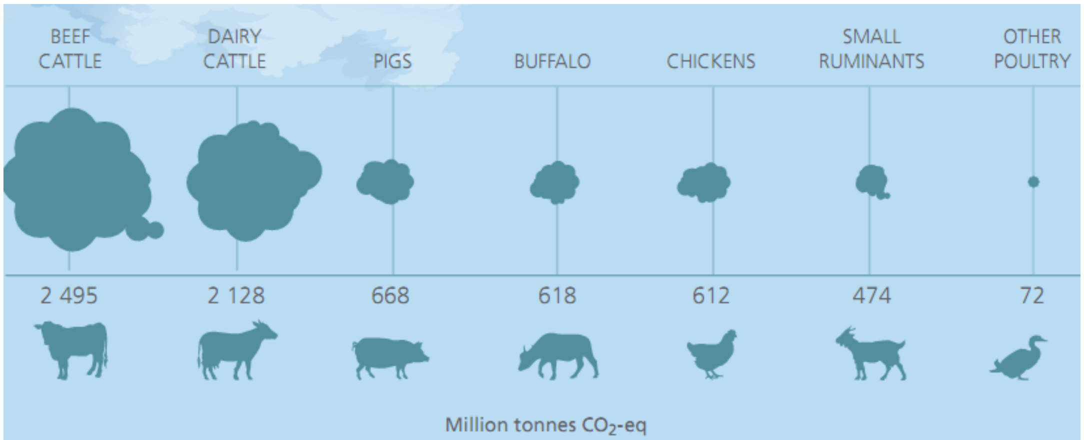


Fig. 7: Emissions are estimated based on commodities.

8.3.5. Manure:

Livestock manure releases CH_4 and N_2O gases. The decomposition of the organic materials in manure releases methane. Manure methane emissions are a function of air temperature, moisture, pH, storage time, and animal diet. Global methane emissions from manure decomposition of 17.5 million tonnes of CH_4 per year. N_2O emissions from manure are dependent on environmental conditions, handling systems, and duration of waste management. Nitrous oxide soil emissions from manure application are the largest source of global N_2O emissions.

8.3.6. Processing and transport

Energy is required for processing animals and their products. Energy use depends on the type of livestock system and if they are small or large scale. More than half of the energy used in confinement systems are for feed production, including seed, herbicides, pesticides, and machinery. Substantial energy is also used for heating, cooling, and ventilation systems. Transportation of livestock products to retailers and transport of feed to livestock farms contribute to GHG emissions. Long distance shipping is the most significant GHG emitter in this category.

8.4. Adaptation and mitigation strategies

Adaptation strategies can improve the resilience of livestock productivity to climate change and involves modification in production and management system based on breeding strategies, institutional and policy changes, science and technology advances, farmer's perception etc. Mitigation measures could significantly reduce the impact of livestock on climate change by reducing GHG emissions through the implementation of different technologies/practices such as carbon sequestration, managing diets to reduce enteric fermentation, improving manure management, and more efficient use of fertilizers. Adaptation and mitigation strategies can make significant impacts if they become part of further research as well as national and regional policies.

8.4.1. Adaptation measures:

8.4.1.1.Modification of Livestock production and management systems.

It involves diversification of livestock animals and crops, integration of livestock systems with forestry and crop production, and changing the timing and locations of farm operations. Diversification of livestock and crop varieties can increase drought and heat wave tolerance, and may increase livestock production when animals are exposed to temperature stress. The diversity of crops and livestock animals is also effective in fighting against climate change-related diseases and pest outbreaks. Shifting locations of livestock and crop production could reduce soil erosion and improve moisture and nutrient retention.

8.4.1.2. Breeding strategies.

Changes in breeding strategies can help animals increase their tolerance to heat stress and diseases and improve their reproduction and growth development. Therefore, the challenge is in increasing livestock production while maintaining the valuable adaptations offered by breeding strategies.

8.4.1.3. Farmers' perception and adaptive capacity.

One of the limiting factors for these changes to succeed is the disposition and capability of farmers to recognize the problem and adopt climate change adaptation and mitigation measures. It is important to collect information about farmers' perceptions to mitigation and adaptation measures and by understanding it; there is a greater chance of accomplishing food security and environmental conservation objectives.

8.4.2. Mitigation measures:

8.4.2.1. Carbon sequestration.

Carbon sequestration can be achieved through decreasing deforestation rates, replanting, higher-yielding climate change adapted crop varieties, improvement of land and water management. Soil organic carbon can be restored in cultivated soils through conservation tillage, erosion reduction, soil acidity management, double-cropping, crop rotations, higher crop residues, mulching and more. Improving pasture management can also lead to carbon sequestration by incorporating trees, improving plant species, legume inter seeding, introducing earthworms, and fertilization.

8.4.2.2. Enteric fermentation.

Enteric fermentation is a source of methane emissions (Fig. 8) that can be reduced through practices such as improvement of animal nutrition and genetics. Mitigating enteric fermentation can be achieved by increasing dietary fat content, providing higher quality forage, increasing protein content, providing feed supplements (e.g. feed antibiotics), use of antimethanogens (vaccines to suppress methane emissions). Providing higher quality forage also results in a reduction of methane emissions because it increases digestibility.

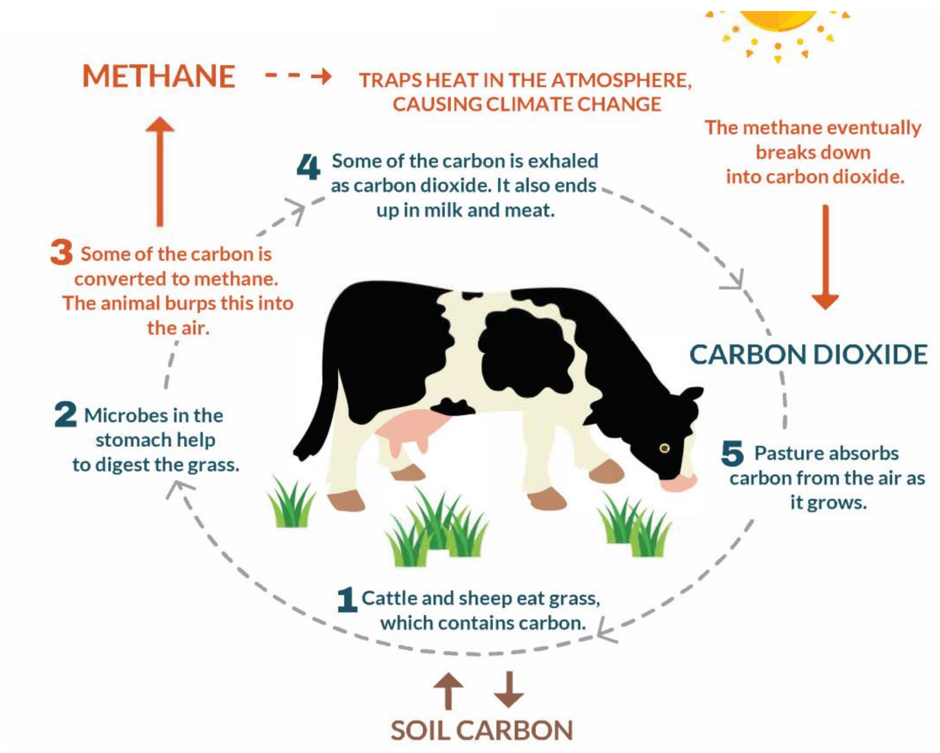


Fig. 8: Methanogenesis in livestock.

8.4.2.3. Manure management.

Most methane emissions from manure management are related to storage and anaerobic treatment. Most mitigation practices involve shortening storage duration, improving timing and application of manure, used of anaerobic digesters, covering the storage, using a solids separator, and changing the animal diets. Anaerobic digestion can reduce methane emissions while producing biogas. Adjusting animal diets can also be used as a mitigation measure, by changing the volume and composition of manure. GHG emissions can be reduced by balancing dietary proteins and feed supplements.

8.4.2.4. Fertilizer management.

Fertilizer application on animal feed crops increases nitrous oxide emissions. Therefore, mitigation measures such as increasing nitrogen use efficiency, plant breeding and genetic modifications, using organic fertilizers, regular soil testing, using technologically advanced fertilizers, and combining legumes with grasses in pasture areas may decrease GHG emissions in feed production.

8.5. Conclusion

In conclusion, climate change will adversely affect livestock production and consequently food security. Livestock production will be negatively impacted and climate change will affect the nutritional content of livestock products, which are one of the suppliers of global calories, proteins and essential micronutrients. Conversely, livestock production also influences climate change. If livestock numbers continue to increase and management practices are not changed, global emissions due to livestock production will continue to increase. Therefore, research on climate change adaptation, mitigation practices, and policy frameworks are critical to protect livestock production under changing climate scenario.

8.6. References

1. Downing M., Nejadhashemi A., Harrigan T., Woznicki S. (2017). Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management*. 16: 145-163.
2. FAO (Food and Agriculture Organization of the United Nations), 2013. Climate-smart agriculture: Sourcebook. FAO, Rome. <http://www.fao.org/3/a-i3325e.pdf>.

3. FAO (Food and Agriculture Organization of the United Nations), IFAD (International Fund for Agricultural Development), WFP (World Food Programme) (2013). The State of Food Insecurity in the World: The Multiple Dimensions of Food Security. FAO, Rome.
4. FAO (Food and Agriculture Organization of the United Nations) (2017). GAEZ - Global Agro-Ecological Zones. <http://www.fao.org/nr/gaez/en/>.
5. IPCC (Intergovermental Panel on Climate Change) (2014). Climate Change 2014: impacts, adaptation, and vulnerability. part A: global and sectoral aspects. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1132.
6. Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S., Bernabucci, U. (2010). Effects of climate change on animal production and sustainability of livestock systems. *Livestock Science*. 130, 57-69.
7. Thornton, P.K., Van de Steeg, J., Notenbaert, A., Herrrero, M. (2009). The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. *Agricultural Systems*. 101, 113-127.

Chapter- 9

Biofloc technology for fish production: Abiotic Stress management

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9.1. Introduction

Aquaculture production is limited by the availability of land and water. Water being a very precious natural resource is dependent on the monsoon rainfall. In India availability of water increase and decreases based on the total percentage of rainfall received during monsoon. With change in climatic conditions it is observed that the monsoon precipitation is changing. To maximally use and reuse the limited water resource in fish production system Biofloc technology comes into play. Presently need for increasing fish production through technological intervention is necessary so as to feed the ever growing population of the country and to be ready for generating food for future to sustain the large population of the country.

Biofloc technology (BFT) recycles water and efficiently utilizes the nutrients through recycling and reusing. BFT is dependent on growth on microbial biomass (biofloc) which maintain water quality by consuming nitrogen compounds and converting it into microbial protein in situ, decreases feed conversion ratio thereby decreases the feed cost (Emerenciano et al., 2013). BFT encourages microbial solids to accumulate in water, quality of which is maintained by active aeration and sufficient mixing of water (Hargreaves, 2013)

9.2. Composition and nutrients in biofloc

Aggregates of algae, bacteria, protozoa and other particulate organic matter such as fecal matter and unutilized feed is known as biofloc. The biofloc is held together by some mucous secretions of the filamentous bacteria or held together by the electrostatic forces. It also has grazers, such as rotifers, ciliates and flagellates protozoa and copepods. The biofloc size may vary from macroscopic to microscopic with size variation from 50 to 200 microns. The dry weight protein content of biofloc ranges from 25-50%, fat content from 0.5 to 15%. It serves as good source of vitamins and minerals (Hargreaves, 2013) (Emerenciano et al., 2013). The control of bacteria community over autotrophic microorganisms is achieved using a high carbon to nitrogen ration (C:N). The nitrogenous by products are easily consumed by the heterotrophic bacteria. The high carbon to nitrogen ratio guarantees optimum heterotrophic bacterial growth and multiplication. The high carbon content restricts algal growth allowing the bacteria to grow. The aerobic microorganisms are efficient in converting feed to new cell material.

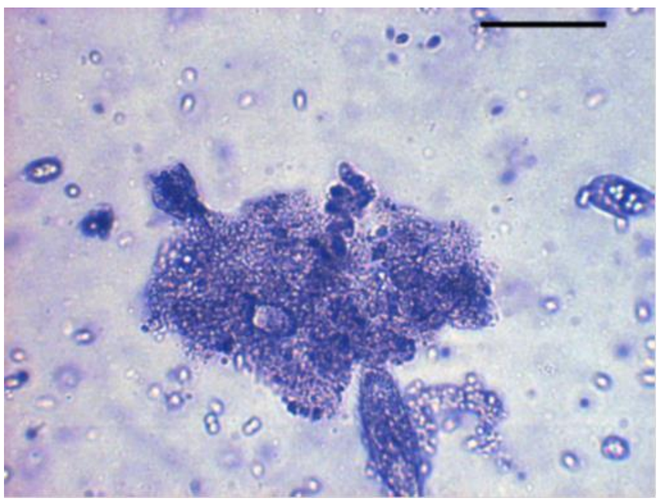


Fig: individual biofloc (100u) (Hargreaves, 2013)

9.3. Sources of Carbon in Biofloc formation

Carbon source acts as substrate for biofloc technology system and for production of microbial cells. The C source needs to be cheap, biodegradable, easily available nearby and be able to get assimilated by bacteria.

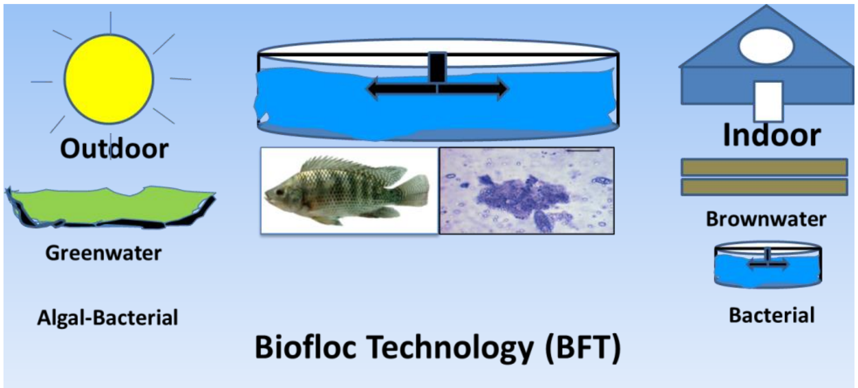
Carbon Source	Microorganism
Acetate	*Macrobrachium rosenbergii*
Cassava meal	*Penaeus monodon*
Cellulose	*Tilapia*
Corn flour	*Hybrid bass and hybrid tilapia*
Dextrose	*Litopenaeus vannamei*

Glycerol and Glycerol + Bacillus	*M. rosenbergii*
Glucose	*M. rosenbergii*
Molasses	*L. vannamei and P. monodon*
Sorghum meal	*Tilapia*
Tapioca	*L. vannamei and M. rosenbergii*
Wheat flour	*Tilapia (O. niloticus)*
Wheat bran + molasses	*Farfantepenaeus brasiensis, F. paulensis and F.duorarum*
Starch	*Tilapia O. niloticus x O. aureus and tilapia (Mozambique)*

Fish species which are able to tolerate high stocking densities, ability to tolerate 3-6mg/l levels of dissolved oxygen, able to tolerate the settling solids in water, high gill filtration, tolerant to higher N in water and are omnivorous with ability to efficiently digest the microbial biofloc are desirable fish species in the Biofloc production system (Emerenciano et al., 2013). Shrimp and Tilapia have been the fish species of choice for BFT due to their physiological adaptations to consume the biofloc and digest the microbial proteins as a source of nutrition. Species intolerant to high solids concentration in wter are not amenable to BFT (Hargreaves, 2013).

9.4. Types of Biofloc Systems:

There are two types of BF systems namely Outdoor and Indoor. This is explained in the below given figure.



9.5. Biofloc Oxygenation

Biofloc requires intensive turbulent mixing of water making the biofloc solids to remain suspended in the water columns all the time. The floc tend to sediment at the bottom in the absence of mixing causing rapid consumption of dissolved oxygen resulting in creating anaerobic zones, release of hydrogen sulphide, methane and ammonia which are detrimental to the health of fish. Solids and sludge is resuspended by repositioning of aerators/paddle aerators. Respiration rate of biofloc water is high other than the fish/shellfish respiration rates. In Indoors, high respiration rates require higher turbulence and mixing of water so as to maintain high dissolved oxygen levels. Higher respiration rates are indicative of shorter response times hence online continuous monitoring and alarm systems are necessary to be installed in Indoor BF systems.

Fig: Biofloc technology commercial-scale (Emerenciano et al., 2013)

Sequential changes in BF formation: In sunlight exposed outdoor BF system time dependent sequential changes are observed. At a certain time interval the algal dominated green water biofloc will get converted into bacteria dominated brown-water biofloc system.

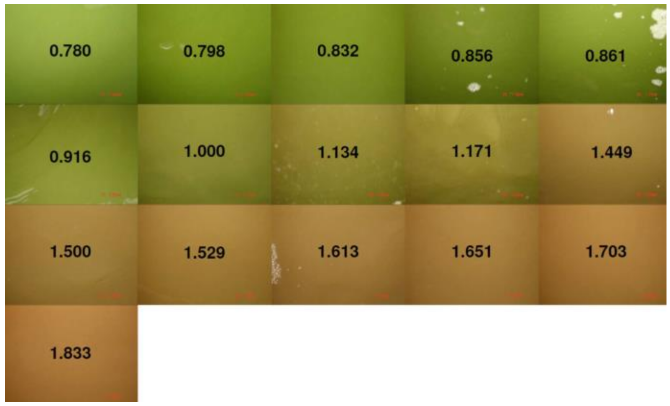
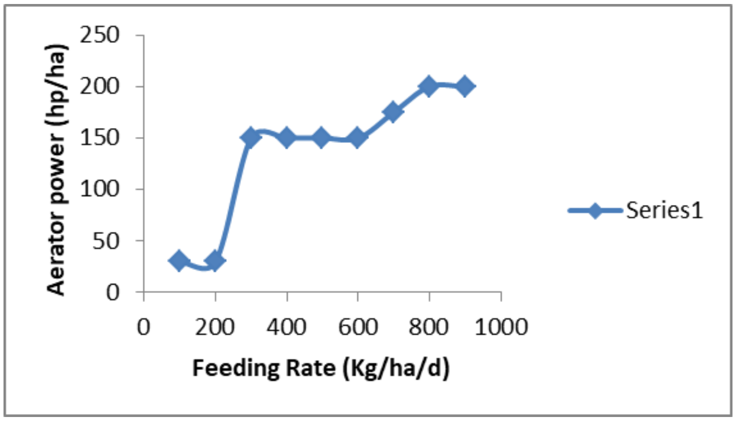


Fig**: The Microbial Community Color Index (MCCI) indicating the transition from an algal to a bacterial system as feed loading increases. The transition between algal and bacterial systems occurs at a feed loading of 300 to 500 kg/ha per day, indicated by an MCCI between 1 and 1.2. (Hargreaves, 2013)**

Feeding Rate (Kg/ha/d)	**Water Color**	**Dominant Pathway**	**Aerator power (hp/ha)**	**Water respiration (mg/L per hr)**	**Net photosynthesis (mg/L per hr)**
100	green	Algae	30	–0.5	+4.2
200	green	Algae	30	–1.0	+8.3
300	green	Algae + Bacteria	150	–5.8	+1.2
400	green	Algae + Bacteria	150	–5.8	–2.0
500	green-brown	Algae + Bacteria	150	–4.0	–1.0
600	brown-green	Bacteria + Algae	150	–4.0	–3.5
700	brown	Bacteria	175	–4.0	–4.0
800	brown	Bacteria	200	–5.0	–5.0
900	brown	Bacteria	200	–6.0	–6.0



9.6. Ammonia Assimilation in BF system:

High intensive aquaculture systems such as BFT requires stringent water quality management. The high levels of ammonia pose toxic threat to the fishes and is to be managed through algal consumption of ammonia, bacterial assimilation of ammonia and nitrification. The complex dynamics of ammonia is interplay between algae and bacterial ammonia utilization. The ammonia assimilation and transformation depends on daily feeding regime, biofloc concentration, ammonia, photo intensity and the presence of carbon: nitrogen ratio. In outdoor BF system algal bloom formation takes place due to plentiful availability of sunlight. The decomposing organic matter from the feed is consumed and stored by algae. The algal density depends on the underwater light intensity, concentration of biofloc solids etc. In algae dominated BFS daily fluctuation in DO and pH even after aeration is predominant. Bacterial BFS is mainly due to heterotrophic bacteria obtaining carbon form organic sources which are limited by the availability of dissolved organic carbon in water. The addition of supplemental carbon source of carbohydrate raises the C: N ratio thereby stimulating bacterial growth. Increase in carbohydrate consumption bacteria create a demand for nitrogen (as ammonia) because organic carbon and inorganic nitrogen are generally taken up in a fixed ratio that reflects the composition and requirement of bacterial cells. Thus the ammonia is controlled by addition of organic carbon which is used by heterotrophic bacteria in the form of bacterial protein. The Heterotrophs grow within a short time of few hours to days. Theses bacterial cells are consumed by fish and serves as supplemental feed. Nitrification occurs in two step oxidation of ammonia, bacteria transforms ammonia to nitrate. Nitrate accumulates overtime in low exchange BFS. Waste nitrogen is repeatedly recycled between dissolved ammonia and solids od algae/bacteria. Removal of solids reduces ammonia.

9.7. Management of BFS

The C:N ratio management is most important in BFS. A feed with 30-35% protein has a C:N ratio of 9-10.1 which is relatively low. Increasing C:N to 12 to 15:1 favors the heterotrophic bacterial pathway of ammonia control. The C:N can be raised by addition of grain pellets, molasses, sugar cane bagasse, chopped hay which are low cost. This simple organic matter is acted rapidly by the here heterotrophic bacteria within minutes to hours. The simple carbohydrates such as sugar or starch and simple sugar are quick. For every 1 kg of 30-38% protein feed an addition of 0.5 to 1 kg of carbohydrate source serves the purpose. Carbohydrate content will increase with increase in the protein content in the feed.

9.8. BF startup

The BF system initially exhibits time lag in peak concentrations of ammonia and then nitrite as different populations of bacteria develop. Startup time depends on temperature, feeding rate schedule, pre seeding with the right kind and quantity of bacteria. Nitrifying bacteria can be separately grown and then seeded in the BF tanks. Adding sludge or water from previously acclimated system is an effective seeding method. Ammonia or nitrate peaks during start-up can be avoided or minimized by adding carbohydrate. 15-20mg/L carbohydrate neutralizes 1mg/l of ammonia. Initial addition of carbohydrate keeps the ammonia low and extends the acclimation time. Once acclimated further supplementation with carbon is optional as the nitrifying bacteria are able to keep ammonia and nitrite at safe concentrations. Carbon addition controls the occasional ammonia spikes.

Fig: Imhoff cones, 25-50 ml/l solid requirement for Tilapia biofloc system

9.9. Solids management

Biofloc typically operates at 500-1000mg/l of suspended solids. A 200-500 mg/l suspended solid concentration is good for BF functionality and controls ammonia without excessive consumption of Dissolve oxygen. A suspended solid concentration of 25-50mg/L is good for tilapia culture system. Solids concentration should be managed as a compromise between the functionality of the biofloc system as a bio-filter (for ammonia control) and the oxygen demand of the water, which increases directly with solids concentration. The SSC be such that the BFS does not require excessive aeration and mixing of water. Optimum solid concentration reduces the risk of depletion of DO, allows natural algal photosynthesis and thereby contributing DO to the system. Solids and Alkalinity Management: Solids can be managed using clarifiers (gravity settling tanks) where by 1-5% water is exchanged. Alkalinity is the capacity of water to buffer or resist changes in pH is response to additions of acids or base. Nitrification leads to loss of alkalinity in biofloc system resulting in drop pH, inhibiting the bacterial growth, accumulates ammonia thereby detrimental to the fish appetite and feeding. This limits daily feeding rate, FCR and finally fish yield. Optimum Alkalinity should be 100-150 mg/L as $CaCO_3$ by regular addition of sodium bicarbonate.

9.10. Advantages and Disadvantages of Biofloc Technology

Due to reduced requirement of water and very less requirement of water exchange BFT improves biosecurity in terms of release of microbial pathogens to the open waters and prevents the escape of high performing fish species such as GIF Tilapia to open waters. It improves feed conversion by making available the feed nitrogen through microbial proteins. BFT enhances water and land use efficiency and water quality. BFT is less sensitive to photoperiod. The disadvantages are that BFT is energy intensive for water mixing and aeration. It has a reduced response time because water respiration rates are elevated. Some startup period is required. Increased instability of nitrification and requires supply of alkalinity. It accumulates nitrates (Emerenciano et al., 2013).

9.11. References

Emerenciano, M., Gaxiola, G., Cuzo, G., 2013. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry, in: Biomass Now - Cultivation and Utilization. InTech. doi:10.5772/53902

Hargreaves, J.A., 2013. Biofloc Production Systems for Aquaculture Southern regional

aquaculture center.

Chapter- 10

Genetic engineering of crop plants for improvement of abiotic stress tolerance in crop pants

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10.1. Introduction

Abiotic stresses such as drought, flood, high or low temperature and salinity are the major cause of poor plant growth, development and crop yield reduction (Acquaah 2012). A combination of two or more abiotic stresses such as drought and heat stress causes more severe crop yield reductions than a single stress (Mittler 2006). With increasing challenges posed by climate change, it is predicted that incident of drought, floods and heat events will be more frequent and severe and it will further reduce crop yields in the tropics and subtropics. Adverse environmental factor leads to morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang *et al.* 2001). Plants try to adopt or mitigate the abiotic stress condition through various mechanisms like early flowering to escape the season of drought, reducing the leaf area (LA), increasing efficiency of roots to acquire water or decreasing activity of stomata, slowing growth, osmotic adjustments and synthesis of antioxidants. Unfortunately, the mechanisms by which crops maintain yield under drought stresses are poorly understood since abiotic stress may individually or in combination at different stages of the plant's development, with different effects on plant function, and thus requires distinct mechanisms for adaptation or tolerance..

It is suggested that stress tolerance mechanisms in a plant are controlled by a variety of genes which are expressed at different stages of plant (Witcombe *et al*. 2008, Fleury *et al.* 2010). Plant adaptations to most abiotic stresses involve a range of traits which combine to contribute plant tolerance. Individual genes have been reported to improve the stress tolerance in some crops, for instance, the transcription factor ZmNF-YB2 has been reported to improve drought tolerance in maize (Nelson *et al*. 2007). This chapter deals with recent advances in understanding mechanisms underlying plants response to various abiotic stresses. The main mechanisms such as signal transduction pathways, regulation of gene expression, ion transport, and detoxification mechanisms are also discussed. Emphasis has been given to transgenic plants that were engineered for abiotic stress tolerance.

10.2. Genes involved in plant's stress perception

Genes involved in stress signal sensing and a cascade of stress-signaling in model plant such as *Arabidopsis thaliana* has been of recent research interest (Winicov and Bastola 1997; Shinozaki and Yamaguchi-Shinozaki 1999). Components of the signal transduction pathway may also be shared by various stress factors such as drought, salt and cold (Shinozaki and Yamaguchi-Shinozaki 1999). Although there are multiple pathways of signal-transduction systems operating at the cellular level for gene regulation, ABA is known component acting in one of the signal transduction pathways, while others act independently of ABA. The early response genes have been known to encode transcription factors that activate downstream delayed response genes (Zhu 2002). Although, specific branches and components exist (Lee *et al*. 2001), the signaling pathways for salt, drought, and cold stresses all interact with ABA, and even converge at multiple steps (Xiong *et al*. 1999). Abiotic stress signalling in plants involves receptor-coupled phospho-relay, phosphoionositol-induced Ca2+ changes, mitogen activated protein kinase (MAPK) cascade, and transcriptional activation of stress responsive genes (Xiong and Zhu 2001). A number of signaling components are associated with the plant response to high temperature, freezing, drought and anaerobic stresses (Grover *et al*. 2001). One of the merits for the manipulation of signaling factors is that they can control a broad range of downstream events that can result in superior tolerance for multiple aspects (Umezawa *et al*. 2006). Alteration of these signal transduction components is an approach to reduce the sensitivity of cells to stress conditions, or such that a low level of constitutive expression of stress genes is induced (Grover *et al*., 1999). Over-expression of functionally conserved At-DBF2 (homolog of yeast DBF2 kinase) showed striking multiple stress tolerance in Arabidopsis plants (Lee *et al*. 1999). Transgenic tobacco plants produced by altering stress signaling through functional reconstitution of activated yeast calcineurin not only opened-up new routes for study of stress signaling, but also for engineering transgenic crops with enhanced stress tolerance (Grover *et al*. 1999). Overexpression of an osmotic-stress-activated protein kinase, SRK2C resulted in a higher drought tolerance in *A. thaliana*, which coincided with the upregulation of stress-responsive genes (Umezawa *et al*. 2004). Similarly, a truncated tobacco mitogen-activated protein kinase kinase kinase (MAPKKK), NPK1, activated an oxidative signal cascade resulting in cold, heat, salinity and drought tolerance in transgenic plants (Kovtun *et al*. 2000, Shou *et al*. 2004). However, suppression of signaling factors could also effectively enhance tolerance to abiotic stress (Wang *et* ***al*. 2005).**

10.3. Genes induced by water stress

Various genes respond to drought-stress in various species, and functions of their gene products have been predicted from sequence homology with known proteins. Many drought-inducible genes are also induced by salt stress and low temperature, which suggests the existence of similar mechanisms of stress responses. Genes induced during drought-stress conditions are thought to function not only in protecting cells from water deficit by the production of impor-tant metabolic proteins but also in the regulation of genes for signal transduction in the drought stress response (Yamaguchi-Shinozaki *et al*. 2002, Shinozaki *et al*. 2003).

10.4. Involvement of genes in transcriptional regulation to enhance water stress tolerance

Transcription factors (TFs) play an important role in activating or deactivating the expression of certain genes. A single gene encoding a specific stress protein does not always result in sufficient expression to produce useful tolerance, because multiple and complex pathways are involved in controlling plant drought responses (Bohnert *et al*. 1995) and because modification of a single enzyme in a biochemical pathway is usually contrasted by a tendency of plant cells to restore homeostasis. Transcription factors typically regulate several genes and are likely to be used extensively in the next generation of genetically modified crops (Yamaguchi-Shinozaki and Shinozaki 1994, Chinnusamy *et al* 2005). Numerous transcriptional regulators are known to be involved in plant responses to drought stress (Yamaguchi-Shinozaki and Shinozaki 2002); most fall into one of the large transcription factor families (AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2 zincfinger, NFY and WRKY); and some cis-elements, bound by these transcription factors, have been identified. For example abscisic acid-responsive elements (ABRE) (Mundy *et al*. 1990) are 50 upstream regions of abscisic acid-responsive genes that are bound by AREB/ABF transcription factors belonging to the basic leucine zipper family. Another cis-element is the dehydration responsive element/C-repeat (DRE/CRT) which is involved in one of the abscisic acid-independent pathways (Yamaguchi-Shinozaki and Shinozaki 1994).

The examples of transcription factor engineering to improve abiotic stress tolerance were overexpression of the ERF/ AP2 factors CBF1, DREB1A and CBF4. Overexpression of these factors resulted in cold, drought and salt tolerance in Arabidopsis (Jaglo-Ottosen *et al*. 1998, Kasuga *et al*. 1999) and it was later shown the similar tolerance could be induced in many crop plants by overexpression of these factors. Numerous transgenic Arabidopsis varieties with improved drought tolerance due to overexpression of various stress-regulated transcription factors have been reported, but similar results have also been obtained in crop plants. Typically a gene coding for a transcription factor in Arabidopsis is isolated, characterized and shown to improve drought response when overexpressed. The gene is then transferred to a crop plant where it often confers the same drought-tolerant phenotype. The HRD gene, coding for an AP2/ERF-like transcription factor) exemplifies this approach. Arabidopsis plants with a gain-of-function mutation in the HRD gene (hrd-D mutants) are drought resistant, salt-tolerant, and overexpress abiotic stress marker genes. Overexpression of the same gene in rice significantly improves water use efficiency both under well-watered conditions (50–100% increase) and under drought (50% increases). These plants also show enhanced photosynthetic assimilation and reduced transpiration). HRD gene overexpression conserves drought tolerance in both dicots and monocots. Various regulatory genes involved in drought tolerance are depicted in Table 1.

10.5. Genes involved in osmotic regulation and ionic balance to enhance water stress tolerance

Genes associated with the synthesis of osmoprotectants have been incorporated into transgenic plants to confer stress-tolerance. Overproduction of compatible solute osmoprotectants such as amino acids such as proline, quaternary and other amines like glycinebetaine and polyamines, and sugars and sugar alcohols such as mannitol, trehalose and galactinol have been achieved in various target plants. Glycine betaine in particular has been extensively studied as a compatible solute, both by genetically engineering its biosynthesis in agriculturally important species and by its exogenous application. When maize plants were transformed with the *betA* gene from Escherichia coli that encodes choline dehydrogenase, they accumulated glycinebetaine in tissues and were more tolerant to drought stress than wild-type plants at different developmental stages. Most importantly their grain yield was 10–23% higher than that of wild-type plants after three weeks of drought stress (Quan *et al*. 2004). In some cases the accumulation of compatible solutes also protects plants against damage by reactive oxygen species (ROS) (Bohnert and Shen 1999); in other cases the solutes have chaperone-like activities that protect other proteins maintaining their structure and function (Diamant *et al*. 2001, McNell *et al*. 1999]. The improvement in drought response was observed in the late vegetative/flowering period as well as the grain-fill period: during these periods, three consecutive days of wilting can reduce grain yield by 30–50%. Stress tolerance conferred by manipulation of cold shock proteins is not only novel, but also appears as a highly promising approach to improving plant productivity in suboptimal growth conditions. Genes encoding enzymes that synthesize osmotic and other protectants are listed in Table 2.

10.6. Improvement of abiotic stress tolerance in crop plants through genetic engineering

Plants have multiple physiological, biochemical and molecular mechanisms to combat with various abiotic stresses. Understanding the mechanisms to perceive and transduce the stress signals to initiate adaptive responses and their engineering using molecular biology and genomic approaches are essential for improving abiotic stress tolerance in crop plants. Several efforts have been made by many researchers for manipulation of genes belonging to diverse categories. Genetic engineering strategies rely on the transfer of one or several genes that are either involved in signaling and regulatory pathways, or that encode enzymes present in pathways leading to the synthesis of functional and structural protectants, or that encode stress tolerance-conferring proteins. Attempts have been made to confer drought resistance to plants through biotechnological approaches and drought tolerant varieties of crops such as rice and soybean have been produced. Several crops have been genetically engineered for improvement of abiotic stress tolerance (Table 3)

Table 1: Regulatory genes involved in drought tolerance

Gene	Gene action	Species	Phenotype	References
ABF3	Transcription factor	Rice	Drought resistance	Oh *et al*., 2005
Alx8	High APX2 and ABA	*Arabidopsis*	Drought resistance	Rossel *et al*., 2006
AREB1	ABRE-dependent ABA signaling	*Arabidopsis*	Drought resistance	Fujita *et al*., 2006
CAbZIP1	Plant development (dwarf phenotype)	*Arabidopsis*	Disease, drought and salt tolerance	Lee *et al*., 2006
CAP2	Transcription factor	Tobacco	Drought and salt tolerance	Shukla *et al*., 2006
DREB	Transcription factor	*Arabidopsis*	Increased tolerance to cold, drought and salinity	Kasuga *et al*., 1999
DREB1	Transcription factor	Rice	Drought, salt and cold tolerance with reduced growth under non-stress	Ito *et al*., 2006
DREB1A	Transcription factor	Tobacco	Drought and cold tolerance	Kasuga *et al*., 2004
DREB2A	Transcription factor	*Arabidopsis*	Drought resistance	Sakuma *et al*., 2006
FAD3 and *FAD8*	Increased fatty acid desaturation	Tobacco	Drought resistance	Meng *et al*., 2005
OsDREB1A	Transcription factor	*Arabidopsis*	Drought, salt, freezing tolerance	Dubouzet *et al*., 2003
OsMYB3R-2	MYB homeodomain, and zinc finger proteins	*Arabidopsis*	Drought, salt, freezing tolerance	Dai *et al*., 2007

TaPP2Ac-1	catalytic subunit (c) of protein phosphatase 2A	Tobacco	Drought resistance; maintain RWC and membrane stability	Xu *et al*., 2007
ZmDREB2A	Encodes HSP & LEA proteins	*Arabidopsis*	Drought and heat tolerance	Qin *et al*., 2007

Table 2: Genes encoding enzymes that synthesize osmotic and other protectants

Gene	Gene action	Species	Phenotype	Reference
Abc	Arg decarboxylase	Rice	Reduced chlorophyll loss under drought stress	Capell *et al*., 1998
Abc	Polyamine synthesis	Rice	Drought resistance	Capell *et al*., 2004
AtTPS1	Trehalose-6-phosphate synthase	Tobacco	Drought resistance; sustained photosyntehsis	Almeida *et al*., 2007
BADH -1	Betaine aldehyde dehydrogenase	Tobacco	Heat tolerance in photosynthesis	Xinghong *et al*., 2005
betA	Choline dehydrogenase (glycinebetaine synthesis)	Maize	Drought resistance at seedling stage and high yield after drought	Ruidang *et al*., 2004
Mt1D	Mannitol-1-phosphate dehydrogenase (mannitol synthesis)	Wheat	Drought and salinity tolerance of calli and plants	Abebe *et al*., 2003
Osm1…Osm4	Osmotin protein accumulation	Tobacco	Drought and salt tolerance in plant water status and proline accumulation	Barthakur *et al*., 2001
otsA	Trehalose-6-phosphate synthase (trehalose synthesis)	Tobacco	photosynthetic activity under drought. Increased carbohydrate accumulation.	Pilon-smits *et al*., 1995
P5CS	Pyrroline carboxylate synthase (proline synthesis)	*Petunia*	Drought resistance and	Yamada *et al*., 2005

			high proline	
P5CS	Pyrroline carboxylate synthase (proline synthesis)	Rice	Increased biomass production under drought and salinity stress	Zhu *et al*., 1998
P5CS	Pyrroline carboxylate synthase (proline synthesis) (tomato	Sugarcane	Drought resistance via antioxidant role of proline	Molinari *et al*., 2007
SMADC	S-adenosylmethioninedecarboxylase (polyamine synthesis)	Tobacco	drought, salinity, Verticillium and Fusarium wilts resistance	Waie and Rajam, 2003
SST/FFT	Fructan accumulation	Potato	Reduced proline accumulation at low water status	Knipp and Honermeier, 2006
TPS1	Trehalose synthesis	Tomato	Drought, salt and oxidative stress tolerance	Cortina and Culianez-
TPS1 and *TPS2*	Trehalose synthesis	Tobacco	Maintenance of water status under drought stress	Karim *et al*., 2007

Table 3: Transgenic crops for water stress tolerance

Pathway targeted	**Gene family**	**Trans gene**	**Transgenic expression**	**Crop**	**Reference**
osmoregulation	H+-PPase	AVP1	CaMV35S	cotton	Pasapula *et al.* 2011
osmoregulation + glycinebetaine biosynthesis	H+-Ppase + choline dehydrogenase	BetA and TsVP	Zm Ubiquitin	maize	Wei *et al.* 2011
ABA biosynthesis	LOS5/ABA3	LOS5	OsHVA22P (stressinducible) and OsActin1	rice	Xiao *et al.* 2009
ABA sensing; farnesyltransferase	farnesyltransferase	BnFTA	RNAi with AtHPR1 promoter (drought induced in shoot)	Canola	Wang *et al.* 2009
		AP37	Os.Cc1 (constitutive)	rice	Oh *et al.* 2009
		CBF3	OsHVA22P	rice	Xiao *et al.* 2009

			(stressinducible) and OsActin1 (constitutive)		
stress response	AP2/ERF	HARDY	CaMV35S	Trifolium alexan-drinum	Abogadallah *et al*. 2011
	NAC	OsNAC10	RCc3 (root) (constitutive expression not efficacious)	rice	Jeong *et al.* 2010
	C2H2-EAR zinc finger protein	ZAT1	OsHVA22P (stressinducible) and OsActin1	rice	Xiao *et al.* 2009, Mittler *et al.* 2006]
	MAP kinase	NPK1	OsHVA22P (stressinducible) and OsActin1	rice	Xiao *et al.* 2009
ion transport	Na+/H+ antiporter	NHX1	Actin1	rice	Xiao *et al.* 2009
	Ser/Thr kinase	SOS2	OsHVA22P (stressinducible)	rice	Xiao *et al.* 2009, Batelli ***et al*.** 2007

10.7. References

1. Abebe T, Guenzi AC, Martin B, Cushman JC (2003). Tolerance of Mannitol-Accumulating Transgenic Wheat to Water Stress and Salinity. Plant Physiol., 131: 1748-1755.
2. Abogadallah G, Nada R, Malinowski R, Quick P (2011). Overexpression of HARDY, an AP2/ERF gene from Arabidopsis, improves drought and salt tolerance by reducing transpiration and sodium uptake in transgenic Trifolium alexandrinum L. Planta., 233:1265-1276.
3. Acquaah G. *Principles of plant genetics and breeding*. John Wiley & Sons; 2009.
4. Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G (2003). Overexpression of a Plasma Membrane Aquaporin in Transgenic Tobacco Improves Plant Vigor under Favorable Growth Conditions but Not under Drought or Salt Stress. Plant Cell, 15: 439-447.

5. Almeida AM, Silva AB, Arajo SS, Cardoso LA, Santos DM, Torn JM, Silva JM, Paul MJ, Fevereiro PS (2007). Responses to water withdrawal of tobacco plants genetically engineered with the AtTPS1 gene: A special reference to photosynthetic parameters. Euphytica, 154: 113-126.
6. Alvim CFC, Cascardo JCM, Nunes CC, Martinez CA, Otoni WC, Fontes EPB (2001). Enhanced accumulation of BiP in transgenic plants confers tolerance to water stress. Plant Physiol., 126: 1042-1054.
7. Araus JL, Bort J, Steduto P, Villegas D, Royo C (2003). Breeding cereals for Mediterranean conditions: Ecophysilogy clues for biotechnology application. Ann. Appl. Biol., 142: 129-141.
8. Bahieldin A, Mahfouz HT, Eissa HF, Saleh OM, Ramadan AM, Ahmed IA, Dyer WE, El-Itriby HA, Madkour MA (2005). Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. Physiol. Plant, 123: 421-427.
9. Barthakur S, Babu V, Bansal KC (2001). Over-expression of osmotin induces proline accumulation and confers tolerance to osmotic stress in transgenic tobacco. J. Plant Biochem. Biotechnol., 10: 31-37.
10. Batelli G, Verslues PE, Agius F, Qiu Q, Fujii H, Pan S, Schumaker KS, Grillo S, Zhu J-K (2007). SOS2 promotes salt tolerance in part by interacting with the vacuolar H+-ATPase and upregulating its transport activity. Molecular and Cellular Biology, 27:7781-7790.
11. Berger B, Parent B, Tester M (2010). High-throughput shoot imaging to study drought responses. Journal of Experimental Botany, 61:3519-3528.
12. Blum A (2011) Drought resistance – is it really a complex trait? Functional Plant Biology, 38:753-757.
13. Bohnert HJ, Nelson DF, Jenson RG (1995). Adaptation to environmental stresses. Plant Cell, 7: 1099-1111.
14. Bohnert HJ, Shen B (1999) Transformation and compatible solutes. Sci. Hortic., 78: 237-260.
15. Boyer JS (1982). Plant productivity and environment. Science, 218:443-448.
16. Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P (1998). Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal

development patterns in vitro and results in putrescine accumulation in transgenic plants. TAG, 97: 246-254.

17. Capell T, Bassie L, Christou P (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc. Natl. Acad. Sci. USA, 101: 9909–9914.
18. Century K, Reuber TL, Ratcliffe OJ (2008). Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. Plant Physiology. 147:20-29.
19. Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M (2002). A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell, 14: 2723–2743.
20. Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. Crop Sci., 45: 437-448.
21. Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetic perspectives on crosstalk and specificity in abiotic stress signalling in plants. J. Experimental Botany 2004;55:225-36.
22. Cortina C, Culiaez-Macia F (2005). Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. Plant Sci., 169: 75–82.
23. Covarrubias AA, Reyes JL (2010). Post-transcriptional gene regulation of salinity and drought responses by plant microRNAs. Plant, Cell & Environment., 33:481-489.
24. Dai X, Xu Y, Ma O, Xu W, Wang T, Xue Y, Chong K (2007). Overexpression of an R1R2R3 MYB Gene, OsMYB3R-2, Increases Tolerance to Freezing, Drought, and Salt Stress in Transgenic Arabidopsis. Plant Physiol., 143: 1739-1751.
25. Diamant S, Eliahu N, Rosenthal D, Goloubinoff P (2001). Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. J. Biol. Chem., 276: 39586–39591.
26. Dubouzet JG, Yoh S, Yusuke K, Mie D, Emilyn G, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). OsDREB genes in rice, (Oryza sativa L.), encode transcription activators that function in
27. Fleury D, Jefferies S, Kuchel H, Langridge P (2010). Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany, 61:3211-3222.

28. Flowers TJ,Yeo AR (1981) Variability in the resistance of sodium chloride salinity within rice(Oryza sativa L.) varieties. New Phytologist, 88:363-373.

29. Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005). AREB1 Is a Transcription Activator of Novel ABRE-Dependent ABA Signaling That Enhances Drought Stress Tolerance in Arabidopsis. Plant Cell, 17: 3470-3488.

30. Gaber, A, Yoshimura K, YamamotoT, Yabuta, Y,Takeda T, Miyasaka H, Nakano Y, Shigeoka S (2006). Glutathione peroxidase-like protein of Synechocystis PCC 6803 confers tolerance to oxidative and environmental stresses in transgenic Arabidopsis. Physiol. Plant, 128: 251-262.

31. Gao SQ, Chen M, Xu ZS, Zhao CP, Li LC, Xu HJ, Tang YM, Zhao X, Ma YZ (2011). The soybean GmbZIP1 transcription factor enhances multiple abiotic stress tolerances in transgenic plants. Plant Molecular Biology, 75:537-553.

32. Grover A, Kapoor A, Satya Lakshmi O, Agrawal S, Sahi C, Katiyar-Agarwal S, Agarwal M, Dubey H (2001). Understanding molecular alphabets of the plant abiotic stress responses. Curr. Sci., 80: 206-216.

33. Grover A, Sahi C, Sanan N, Grover A (1999). Taming abiotic stresses in plants through genetic engineering: Current strategies and perspective. Plant Sci., 143: 101-111.

34. Hideg NT, Oberschall A, Dudits D, Vass I (2003) Detoxification function of aldose/aldehyde reductase during drought and ultraviolet-B (280-320 nm) stresses. Plant Cell Environ., 26: 513-522.

35. Hirayama T, Shinozaki K (2010). Research on plant abiotic stress responses in the post-genome era: past, present and future. The Plant Journal, 61:1041-1052.

36. Huang X-Y, Chao D-Y, Gao JP, Zhu M-Z, Shi M, Lin HX(2009). A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes & Development, 23:1805-1817.

37. Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol., 47: 141-153.

38. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001). Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J., 27: 325-333.
39. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998). Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance, Science, 280: 104-106.
40. Jang JY, Lee SH, Rhee JY, Chung GC, Ahn SJ, Kang H (2007). Transgenic Arabidopsis and tobacco plants overexpressing an aquaporin respond differently to various abiotic stresses. Plant Mol. Biol., 64: 621-632.
41. Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Do Choi Y, Kim M, Reuzeau C, Kim JK (2010). Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiology, 153:185-197.
42. Joshi-Saha A, Valon C, Leung J (2011). Abscisic acid signal off the STARTing block. Molecular Plant, 4:562-580.
43. Karim S, Aronsson H, Ericson H, Pirhonen M, Leyman B, Welin B, Palva ET, Patrick VD, Kjell-Ove H (2007). Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. Plant Mol. Biol., 64: 371-386.
44. Kasuga M, Miura S, Shinozaki K, Kazuko Y-S (2004). A Combination of the rabidopsis DREB1A Gene and Stress-Inducible rd29A Promoter Improved Drought- and Low-Temperature Stress Tolerance in Tobacco by Gene Transfer. Plant Cell Physiol., 45: 346-350.
45. Kasuga M, Qiang L, Miura S, Shinozaki K Y, Shinozaki K, Liu Q (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnol., 17: 287-291.
46. Katiyar-Agarwal S, Agarwal M, Grover A (2003). Heat-tolerant basmati rice engineered by over-expression of hsp101. Plant Mol. Biol., 51:677–686.
47. Kline KG, Sussman MR, Jones AM(2010). Abscisic acid receptors. Plant Physiology, 154:479-482.

48. Knipp G, Honermeier B (2006). Effect of water stress on proline accumulation of genetically modified potatoes (Solanum tuberosum L.) generating fructans. J. Plant Physiol., 163: 392-397.

49. Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci

50. Larkindale J, Hall JD, Knight MR, Vierling E (2005). Heat Stress Phenotypes of Arabidopsis Mutants Implicate Multiple Signaling Pathways in the Acquisition of Thermotolerance. Plant Physiol., 138:882-897.

51. Lee JH, van Montagu M, Verbruggen N (1999). A highly conserved kinase is an essential component for stress tolerance in yeast and plant cells. Proc. Natl. Acad. Sci., USA, 96: 5873-5877.

52. Lee SC, Choi HW, Hwang IS, Choi DS, Hwang BK (2006). Functional roles of the pepper pathogen-induced bZIP transcription factor, CAbZIP1, in enhanced resistance to pathogen infection and environmental stresses. Planta, 224: 1209-1225.

53. Li G-L, Chang H, Li B, Zhou W, Sun D-Y, Zhou R-Z (2007). The roles of the at DjA2 and at DjA3 molecular chaperone proteins in improving thermotolerance of Arabidopsis thaliana seedlings. Plant Sci., 173:408-416.

54. Lv S-L, Lian L-J, Tao P-L, Li Z-X, Zhang K-W, Zhang J-R (2009). Overexpression of Thellungiella halophila H+-PPase (TsVP) in cotton enhances drought stress resistance of plants. Planta, 229:899-910.

55. Maqbool SB, Zhong H, El-Maghraby Y, Ahmad A, Chai B, Wang W, Sabzikar R, Sticklen MB (2002). Competence of oat (*Avena sativa* L.) shoot apical meristems for integrative transformation, inherited expression, and osmotic tolerance of transgenic lines containing hva1. TAG, 105: 201-208.

56. Markus, KG , Markus Suh, Su JS, Kolukisaoglu A, Louis P, Sonia C, Mark D, Richter A, Weder B, Schulz B, Martinoia E (2004). Disruption of AtMRP4, a guard cell plasma membrane ABCC-type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility. Plant J., 39: 219-236.

57. McNeil SD, Nuccio ML, Hanson AD (1999). Betaines and related smoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol., 120: 945-949.

58. Meng Z Barg R, Yin M, Gueta-Dahan Y, Leikin-Frenkel A, Salts Y, Shabtai S, Ben-Hayyim G (2005). Modulated fatty acid desaturation via overexpression of two distinct ?-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. Plant J., 44: 361-371.
59. Mittler R (2006). Abiotic stress, the field environment and stress combination. Trends in Plant Science, 11:15-19.
60. Molinari HBC, Marur CJ, Daros E, Freitas de Campos MK, Portela de Carvalho JFR, Filho JCB, Pereira LFP, Vieira LGE (2007). Evaluation of the stress-inducible production of proline in transgenic sugarcane (Saccharum spp.): Osmotic adjustment, chlorophyll fluorescence and oxidative stress. Physiol. Plant., 130: 218-229.
61. Molnar I, Gáspár L, Sárvári E, Dulai S, Hoffmann B, Molnár-Láng M, Galiba G (2004) Physiological and morphological responses to water stress in *Aegilops biuncialis* and *Triticum aestivum* genotypes with differing tolerance to drought. Func Plant Biol 31:1149–1159
62. Morran S, Eini O, Pyvovarenko T, Parent B, Singh R, Ismagul A, Eliby S, Shirley N, Langridge P, Lopato S (2011). Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. Plant Biotechnology Journal, 9:230-249.
63. Morison JIL, Baker NR, Mullineaux PM, Davies WJ (2008). Improving water use in crop production. Philosophical Transactions of the Royal Society B:Biological Sciences, 363:639-658.
64. Mundy J, Yamaguchi-Shinozaki K, Chua NH (1990). Nuclear proteins bind conserved elements in the abscisic acid-responsive promoter of a rice rab gene. Proc. Natl. Acad. Sci. USA, 87: 406-410.
65. Myouga F, Motohashi R, Kuromori T, Nagata N, Shinozak K (2006). An Arabidopsis chloroplast-targeted Hsp101 homologue, APG6, has an essential role in chloroplast development as well as heat-stress response. Plant J., 48: 249-260.
66. Nakajima N, Itoh T, Takikawa S, Asai N, Tamaoki M, Aono M, Kubo A, Azumi Y, Kamada H, Saji H (2002). Improvement in ozone tolerance of tobacco plants with an antisense DNA for 1-aminocyclopropane-1- carboxylate synthase. Plant Cell Environ., 25: 727-735.

67. Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009). Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiology, 149:88-95.

68. Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG *et al*. (2007). Plant nuclear factor Y(NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. In Proceedings of the National Academy of Sciences, 104:16450-16455.

69. Oh S-J, Kim YS, Kwon C-W, Park HK, Jeong JS, Kim J-K (2009) Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physiology, 150:1368-1379.

70. Park BJ, Liu Z , Kanno A , Kamey T (2005a). Genetic improvement of Chinese cabbage for salt and drought tolerance by constitutive expression of a B. napus LEA gene. Plant Sci., 169: 553-558.

71. Park BJ, Liu Z, Kanno A, Kamey T (2005b). Increased tolerance to salt and water-deficit stress in transgenic lettuce (Lactuca sativa L.) by constitutive expression of LEA. Plant Growth Regul., 45: 165-171.

72. Pasapula V, Shen G, Kuppu S, Paez-Valencia J, Mendoza M, Hou P, Chen J, Qiu X, Zhu L, Zhang X *et al*. (2011). Expression of an Arabidopsis vacuolar H+-pyrophosphatase gene (AVP1) in cotton improves drought- and salt tolerance and increases fibre yield in the field conditions. Plant Biotechnology Journal, 9:88-99

73. Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Kazuko Y-S, Hoisington D (2004). Stress-induced expression in wheat of the Arabidopsis thaliana DREB1A gene delays water stress symptoms under greenhouse conditions. Genome, 47: 493-500.

74. Peng Y, Lin W, Cai W, Arora R (2007). Overexpression of a *Panax ginseng* tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic Arabidopsis plants. Planta, 226: 729-740.

75. Pilon-Smits EHA, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek PJ, Smeekens SCM (1995). Improved performance of transgenic fructanaccumulating tobacco under drought stress. Plant Physiol., 107: 125-130.

76. Qin F, Kakimoto M, Sakuma Y, Maruyama K, Osakabe Y, Lam-Son Phan Tran L-Sp, Shinozaki K, Yamaguchi-Shinozaki K (2007). Regulation and functional analysis of ZmDREB2A in response to drought and heat stresses in Zea mays L Plant J., 50: 54-69.
77. Rhoads DM, Samuel JW, You Z, Mrinalini M, Elthon TE (2005). Altered gene expression in plants with constitutive expression of a mitochondrial small heat shock protein suggests the involvement of retrograde regulation in the heat stress response. Physiol. Plant, 123:435-444.
78. Rodrigues SM, Andrade MO, Gomes APS, DaMatta FM, Baracat-Pereira MC, Fontes EPB (2006). Arabidopsis and tobacco plants ectopically expressing the soybean antiquitin-like ALDH7 gene display enhanced tolerance to drought, salinity, and oxidative stress. J. Exp. Bot., 57: 1909-1918.
79. Rohila JS, Jain RK, Wu R (2002). Genetic improvement of Basmati rice for salt and drought tolerance by regulated expression of a barley Hva1 cDNA. Plant Sci., 163: 525-532.
80. Rossel JB, Philippa BW, Luke H, Soon CW, Andrew P, Philip MM, Barry JP (2006). A Mutation Affecting Ascorbate Peroxidase 2 Gene expression reveals a link between responses to high light and drought tolerance. Plant Cell Environ., 29: 269-281.
81. Ruidang Q, Mei S, Hui Z, Yanxiu Z, Juren Z (2004). Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. Plant Biotechnol. J., 2: 477-486.
82. Sakuma Y, Maruyama K, Osakabe Y, Qina F, Seki M, Shinozaki K, Kazuko Y-S (2006). Functional Analysis of an Arabidopsis Transcription Factor, DREB2A, Involved in Drought-Responsive Gene Expression. Plant Cell, 18: 1292-1309.
83. Salekdeh GH, Reynolds M, Bennett J, Boyer J (2009). Conceptual framework for drought phenotyping during molecular breeding. Trends in Plant Science, 14:488-496.
84. Seo JS, Joo J, Kim MJ, Kim YK, Nahm BH, Song SI, Cheong JJ, Lee JS, Kim JK, Do Choi Y (2011). OsbHLH148, a basic helix-loop-helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. Plant Journal, 65:907-921.
85. Shinozaki K, Yamaguchi-Shinozaki K (1999). Molecular responses todrought stress. In: Shinozaki K, Yamaguchi-Shinozaki K (eds) Molecular responses to cold, drought, heat and salt stress in higher plants. R.G. Landes Co., Austin, pp. 11-28.

86. Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Current Opinion in Plant Biology, 6:410-17.

87. Shou H, Bordallo P, Wang K (2004). Expression of the Nicotiana protein kinase (NPK1) enhanced drought tolerance in transgenic maize. J. Exp. Bot., 55: 1013-1019.

88. Shukla RK, Raha S, Tripathi V, Chattopadhyay D (2006). Expression of CAP2, an APETALA2-Family Transcription Factor from Chickpea, Enhances Growth and Tolerance to Dehydration and Salt Stress in Transgenic Tobacco. Plant Physiol., 142: 113-123.

89. Skirycz A, Vandenbroucke K, Clauw P, Maleux K, De Meyer B, Dhondt S, Pucci A, Gonzalez N, Hoeberichts F, Tognetti VB *et al*. (2011). Survival and growth of Arabidopsis plants given limited water are not equal. Nature Biotechnology, 29:212-214.

90. Song S-Y, Chen Y, Chen J, Dai X-Y, Zhang W-H (2011) Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress. Planta, 234:331-345.

91. Tarczynski MC, Jensen RG, Bohnert HJ (1993). Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science, 259: 508-510.

92. Tardieu F, Tuberosa R (2010). Dissection and modelling of abiotic stress tolerance in plants. Current Opinion in Plant Biology, 13:206-212.

93. Umezawa T, Yoshida R, Maruyama K, Yamaguchi-Shinozaki K, Shinozaki K (2004). SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA, 101:17306-17311.

94. Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006a). Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Current Opinion in Biotechnology, 17:113-122.

95. Umezawa T, Okamoto M, Kushiro T, Nambara E, Oono Y, Seki M, Kobayashi M, Koshiba T, Kamiya Y, Shinozaki K (2006b). CYP707A3, a major ABA 8'-hydroxylase involved in dehydration and rehydration response in Arabidopsis thaliana. Plant J., 46: 171-182.

96. Waie B, Rajam MV (2003). Effect of increased polyamine biosynthesis on stress responses in transgenic tobacco by introduction of human S-adenosylmethionine gene. Plant Sci., 164: 727-734.

97. Wang P, Duan W, Takabayashi A, Endo T, Shikanai T, Ji-Yu Ye, Hualing Mi (2006). Chloroplastic NAD(P)H Dehydrogenase in Tobacco Leaves Functions in Alleviation of Oxidative Damage Caused by Temperature Stress. Plant Physiol., 141: 465-474.

98. Wang T, Chen L, Zhao M, Tian Q, Zhang W-H (2011). Identification of drought-responsive microRNAs in Medicago truncatula by genome-wide high-throughput sequencing. BMC Genomics, 12:367.

99. Wang WX, Vinocur B, Shoseyov O, Altman A (2001) Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. Acta Hort; 560:285-92.

100. Wang Y, Beaith M, Chalifoux M, Ying J, Uchacz T, Sarvas C, Griffiths R, Kuzma M, Wan J, Huang Y (2009)/ Shoot-specific downregulation of protein farnesyltransferase (alpha-subunit) for yield protection against drought in canola. Molecular Plant, 2:191-200.

101. Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT *et al* (2005). Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J., 43: 413-424.

102. Wang G-P, Hui Z, Li F, Zhao M-R, Zhang J, Wang W (2010). Improvement of heat and drought photosynthetic tolerance in wheat by overaccumulation of glycinebetaine. Plant Biotechnology Reports, 4:213-222.

103. Wei A, He C, Li B, Li N, Zhang J (2011).: The pyramid of transgenes TsVP and BetA effectively enhances the drought tolerance of maize plants. Plant Biotechnology Journal, 9:216-229.

104. Winicov I, Bastola DR (1997). Salt tolerance in crop plants: New approaches through tissue culture and gene regulation. Acta Physiol. Plant, 19: 435-449.

105. Witcombe JR, Hollington PA, Howarth CJ, Reader S, Steele KA (2008). Breeding for abiotic stresses for sustainable agriculture. Philosophical Transactions of the Royal Society B:Biological Sciences, 363:703-716.

106. Xiao B, Huang Y, Tang N, Xiong L (2007). Over-expression of a LEA gene in rice improves drought resistance under the field conditions. TAG, 115: 35-46.

107. Xiao B-Z, Chen X, Xiang C-B, Tang N, Zhang Q-F, Xiong L-Z (2009). Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Molecular Plant, 2:73-83.

108. Xinghong Y, Zheng L, Congming L (2005). Genetic Engineering of the Biosynthesis of Glycinebetaine Enhances Photosynthesis against HighTemperature Stress in Transgenic Tobacco Plants. Plant Physiol., 138: 2299-2309.

109. Xiong L, Ishitani M, Zhu J-K (1999). Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in Arabidopsis. Plant Physiol., 119: 205-211.

110. Xiong L, Zhu JK (2001). Plant abiotic stress signal transduction: molecular and genetic perspectives. Physiol. Plant, 112: 152-166.

111. Xu C, Jing R, Mao X, Jia X, Chang X (2007). A Wheat (*Triticum aestivum*) Protein Phosphatase 2A Catalytic Subunit Gene Provides Enhanced Drought Tolerance in Tobacco. Ann. Bot., 99: 439-450.

112. Xu Z-S, Chen M, Li L-C, Ma Y-Z (2011). Functions and application of the AP2/ERF transcription factor family in crop improvement. Journal of Integrative Plant Biology, 53:570-585.

113. Xu M, Li L, Fan Y, Wan J, Wang L (2011). ZmCBF3 overexpression improves tolerance to abiotic stress in transgenic rice (*Oryza sativa*) without yield penalty. Plant Cell Reports,1-9.

114. Yamada M, Morishita H, Urano K, Shiozaki N, Kazuko Y-S, Shinozaki K, Yoshiba Y (2005). Effects of free proline accumulation in petunias under drought stress. J. Exp. Bot., 56: 1975-1981.

115. Yamaguchi-Shinozaki K, Kasuga M, Liu Q, Nakashima K, Sakuma Y, Abe H, Shinwari ZK, Seki M, Shinozaki K (2002) Biological mechanisms of drought stress response. JIRCAS Working Rep. 45:1–8.

116. Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Kiyosue T, Shinozaki K (1994). Function and regulation of genes that are induced by dehydration stress in Arabidopsis thaliana. JIRCAS J., 1: 69-79.

117. Yang J-V, Sun Y, Sun A-G, Yi S-Y, Qin J, Li M-H, Liu J (2006). The involvement of chloroplast HSP100/ClpB in the acquired thermotolerance in tomato. Plant Mol. Biol., 62: 385-395.

118. Yang S, Vanderbeld B, Wan J, Huang Y (2010). Narrowing down the targets: towards successful genetic engineering of drought tolerant crops. Molecular Plant, 3:469-490.

119. Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang ZY (2005). Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (Medicago sativa). Plant J., 42: 689-707.

120. Zhang JZ, Creelman RA, Zhu JK (2004). From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. Plant Physiol., 135: 615-621.

121. Zhang S-W, Li C-H, Cao J, Zhang Y-C, Zhang S-Q, Xia Y-F, Sun DY, Sun Y (2009). Altered architecture and enhanced drought tolerance in rice via the down-regulation of indole-3-acetic acid by TLD1/OsGH3.13 activation. Plant Physiology, 151:1889-1901.

122. Zhao HW, Chen YJ, Hu YL, Gao Y, Lin ZP (2000). Construction of a trehalose-6-phosphate synthase gene driven by drought responsive promoter and expression of drought-resistance in transgenic tobacco. Acta Bot. Sin., 42: 616-619.

123. Zheng X, Chen B, Lu G, Han B (2009). Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochemical and Biophysical Research Communications, 379:985-989.

124. Zhu BC, Su J, Chan MC, Verma DPS, Fan YL, Wu R (1998). Overexpression of a Delta (1)-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water- and salt-stress in transgenic rice. Plant Sci. 139: 41-48.

125. Zhu JK (2002). Salt and drought stress signal transduction in plants.Annu. Rev. Plant Biol., 53: 247-273.

126. Zhu J, Ingram PA, Benfey PN, Elich T (2011). From lab to field, new approaches to phenotyping root system architecture. Current Opinion in Plant Biology, 14:310-317.

Chapter- 11

Soil health assessment and management framework

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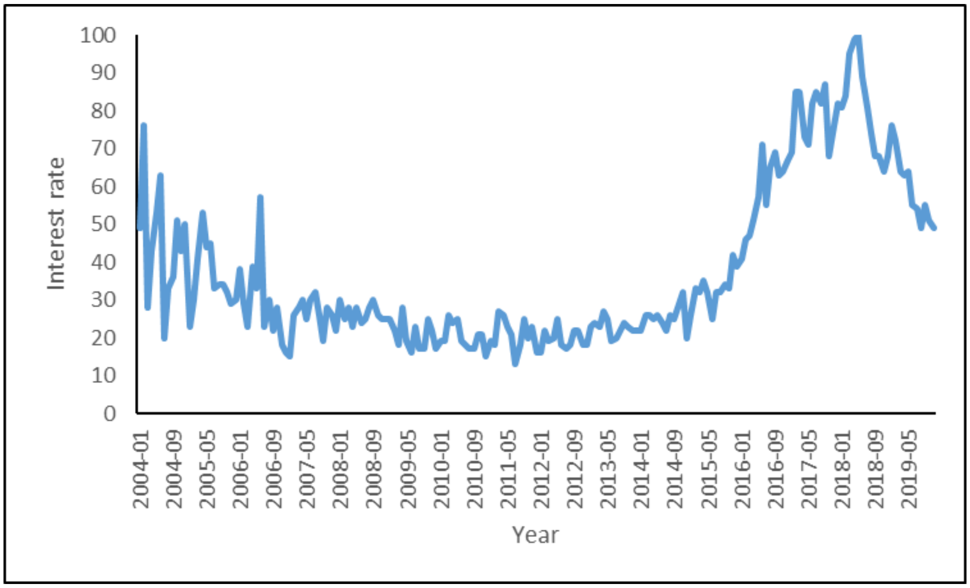
11.1. Introduction

The term soil health has gained lot of popularity in last five years as shown by google trend analysis from internet (Fig.1). it has also shown that soil health word is a buzz word in India for last few years and has given utmost important by the government and policy makers. Therefore, it is very important to understand the concept of soil health, its assessment framework and management of soil health.



11.2. To understand soil health, lets first understand what soil is?

There are many definitions depending upon its utility by different workers. For eg. Soil is the thin upper layer of earth for geologist, Engineers consider soil as earthy material which can be manipulated for construction whereas for farmers it is the medium for plant growth and for agricultural scientists it is not only medium for plant growth but also a solution to climate change due to its high potential for carbon sequestration and maintenance of water, air and environmental quality to sustain plant, animal and human life.

The first definition of soil was given by Russian soil scientist Dokuchaev in the early 1800. According to his definition, soil as a natural body developed from parent material in response to five soil forming factors; climate, vegetation, parent material, topography, and time. Then it is revised several times by many scientists and the modern definition of soil recognized by Soil Survey Staff (2010) of USDA "soil is a natural body comprised of soil (mineral and organic matter), liquids and gases that occur on the land surfaces and occupies space and is characterized by one or both of the following: horizons or layers that are different from initial matter as a result of additions, losses transfer and transformations of energy and matter or the ability to support rooted plants in natural environment. This definition of soil provides a strong definition of soil in terms of its use and genesis. Soil is a non-renewable natural resource and plays crucial role in sustaining life on earth by providing several ecosystem services.

11.3. As per Blum (2014), the functions of soil are given below

- biomass production, including agriculture and forestry,
- storing, filtering and transforming nutrients, substances and water,
- biodiversity, such as habitats, species and genes,
- physical and cultural environment for humans and human activities,
- source of raw materials,
- acting as carbon pool, and
- archive of geological and archaeological heritage.

The soil health of a particular area is judged depending upon the performance of soil to deliver these seven services in terms of several indicators. But before going into deeper aspects of soil health, lets understand the definition of soil health "the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals and humans" (Natural Resources Conservation Service – USDA-NRCS, 2012; Soil Renaissance, 2014). So, soil health supports the idea of ecosystem in which every organism has a crucial role to play for sustenance and maintenance of soils ability to function optimally. for many people the idea of soil health is similar to soil quality, but there is a little difference between these concepts. According to Doran and Parkin (1994), soil quality is capacity of soil to function within ecosystem and land use boundaries, to sustain productivity, maintain environmental quality, and promote plant and animal health." Soil quality has two parts

- **Inherent soil quality** – it depends upon the soils natural composition and properties (like texture, soil type) influenced by long term factors and processes of soil formation. It generally does not change by changing agronomic management practices.
- **Dynamic soil quality** – it refers to change in soil quality due to change in soil use and management practices over a period of time. In other words, we can say that dynamic soil quality is equivalent to soil health.

In simple words, we can define soil health as soil functions driven by soil biota. In soil health concept, the quantity and quality of soil biological diversity are the key drivers of soil health. It is because the soil biota is responsible for making soil a living identity. These organisms in the soil are responsible for 90 % of soil biogeochemical cycling of nutrients and recycling of organic matter in soil. The key functions of soil biota are (Moebius-Clune *et al.*, 2016) -

- Decompose organic matter
- Sequester carbon
- recycle, store(immobilize), and release (mineralize) nutrients for sustained availability to plants
- Fix nitrogen
- Stabilize and maintain soil structure
- Biologically suppress plant pest
- Parasitizes and damage plant
- Soil compaction
- Promote plant growth

11.4. Detoxify pollutants and clean water

This shows the enormous role of soil biota in maintaining soil health. In essence, soil biota are managers of healthy soil. The overall idea for evolution of soil health is nicely present in

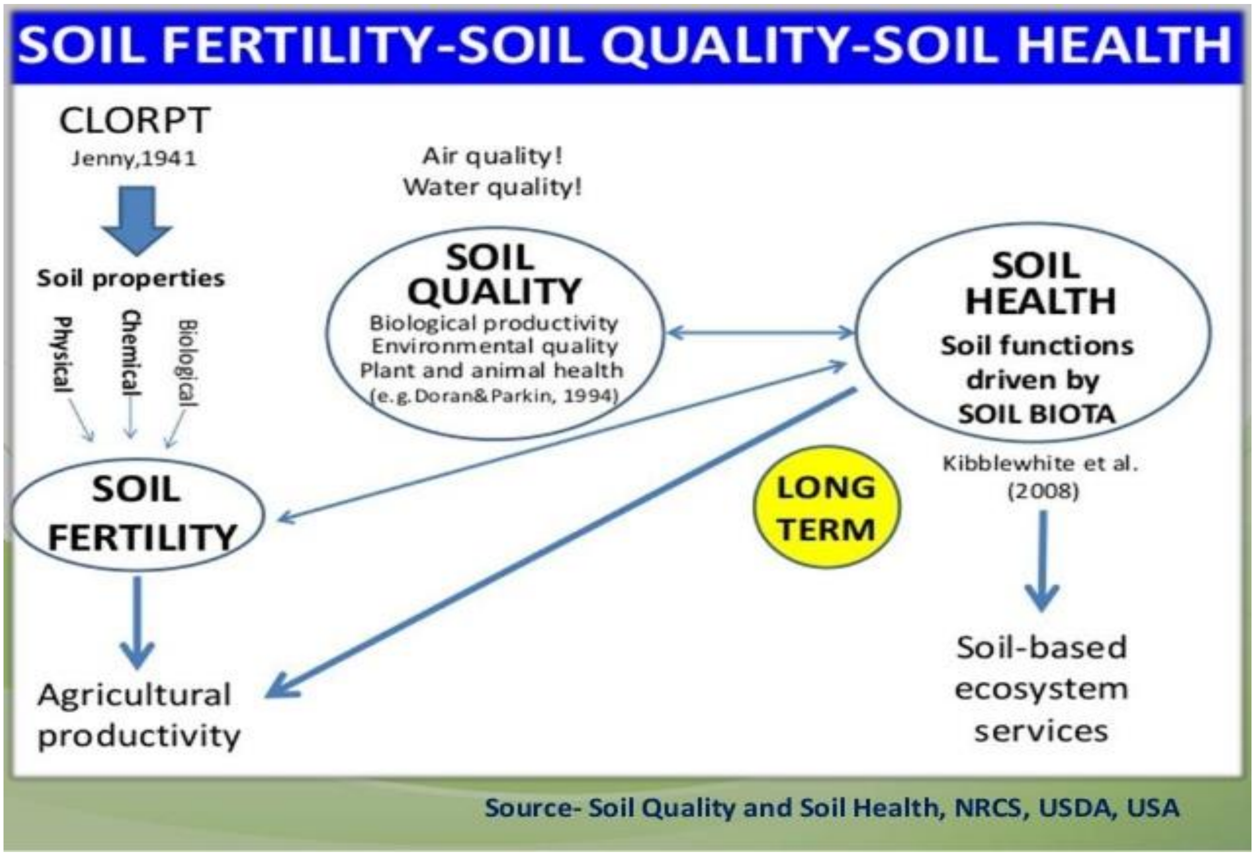


Fig.2 the evolution of idea of soil health (Adapted from **https://www.slideshare.net/FAOoftheUN/edmundo-barrios**)

In fig. 2 the whole idea of evolution of soil fertility, soil quality and soil health is presented here. The soil forming factors were described by Jenny in 1941 which affects soil physical, chemical and biological properties. This leads to the idea of soil fertility as the inherent capacity of soil to supply sufficient quantity of nutrients at right time for sustained agricultural productivity. It means that physical, chemical and biological properties of soil play important role in maintaining soil fertility. But over a period of time, it was observed that agricultural production systems generate a lot of greenhouse gases (24 % of total GHG emission) and contributes to climate change and global warming. Therefore, the idea of environmental quality including air and water quality has been introduced in the concept of soil quality by Doran and Parkin (1994). According to FAO to achieve healthy soil, we need to focus on the 10 main threats to soil functions: soil erosion, soil organic carbon loss, nutrient imbalance, soil acidification, soil contamination, waterlogging, soil compaction, soil sealing, salinization and loss of soil biodiversity. To address these issues, the concept of soil health focuses on ecosystem approach and the main drivers of healthy soils are soil biota to deliver soil-based ecosystem services.

11.5. Benefits of maintaining healthy soil

- Better plant growth, yield and quality
- Reduced risk of yield loss during periods of environmental stress (heavy rain, pest or disease outbreak)
- Better field access during wet periods
- Reduced fuel cost by requiring less tillage
- Reduced input cost by decreasing losses, and improving use efficiency of fertilizer, Pesticides, herbicide and irrigation application

11.6. Characteristics of healthy soil ((Moebius-Clune *et al.*, 2016) –

- Good soil tilth- crumby, well-structured, dark with organic matter
- Sufficient soil depth
- Good water storage and good drainage
- Sufficient supply of nutrients, but not excess
- Small population of plant pathogens and insect pests
- Large population of beneficial microorganism
- Low weed pressure
- Free of chemicals and toxins that may harm the crop
- Resistant to degradation
- Resilience when unfavorable condition occurs

11.7. Common soil constraints

To maintain healthy soil, it is important to understand and recognize soil constraints that reduces crop yields, farm income and environmental quality. The important soil constraints are discussed below-

11.7.1. Soil compaction

Compaction can occur at surface and sub-surface soils due to heavy traffic or tillage when the soil is excessively wet. The other factors are uncontrolled traffic and heavy loads of machinery which creates compact soil layers in subsurface layers.

11.7.1.1. Effect of compact layer on crop productivity

- Reduced rooting system in surface and subsurface soils
- Low water infiltration rate, resulting in more runoff, soil erosion, waterlogging and poor aeration
- Increased sensitiveness to drought due to reduced water storage capacity and rooting depth
- Increased incidence of plant diseases and more biotic stress to plants
- Reduced nutrient access due to poor root growth and restricted water flow
- Increased cost of tillage and yield losses

11.7.1.2. Poor soil aggregation

Poor aggregated soils have higher risks of runoff and erosion which increases risk of loss in crop productivity. Aggregates are formed when mineral and organic particles bind together. The factors contributing for poor aggregation are intensive tillage, lack of cover cropping, low active rooting density, shallow rooted crops, limited during of crops in the field, limited additions of different sources of organic amendments like farm yard manure, compost etc. in the soil, low biological activity to stabilize soil aggregates

11.7.1.3. Effect of poor aggregation

- Poor seedling emergence and stand establishment
- Cracking and crusting
- Poor water infiltration and storage
- Increased occurrence of erosion and runoff
- Reduced root growth
- Less active microbial communities
- Reduced aeration
- Reduced drought resistance due to reduced water intake

11.8. Weed pressure

When plants are unhealthy and weak then they are less able to defend themselves against pathogens and weeds. The main factors responsible for high weed pressure are poor crop rotations and omission of cover crops, increased resistance to herbicide due to use of same kind of molecule continuously for several years

11.8.1. Effect of weed pressure

- Poor crop establishment, reduced growth, reduced yield and poor quality of produce
- Increased disease and pest damage
- Increased cost of weed control
- Interference with cultural practices and harvest

11.9. Heavy metal contamination

Heavy metal contamination may occur due to presence of heavy metal bearing parent material in the soil or due to use of sewage and sludge from high loading of heavy metals in the field. It may also occur due to industrial leakage or pesticides and fertilizer application containing heavy metals in it. Heavy metals negatively impact all the functions of soil.

11.9.1. Effect of heavy metal contamination

- Inhibition of biological activity
- Poor crop germination and growth negatively impacting crop quality and yield reduction

11.10. Low water and nutrient retention

Lower content of soil organic matter causes poor soil structure and low water holding capacity reducing nutrient mobility and plant growth. Intensive tillage, mono-cropping, lack of adaption of cover cropping, green manuring and applications of organic soil amendments.it can result in ground and surface water pollution, reduced microbial community, nutrient deficiency and poor plant growth and drought stress.

11.11. Salinity and sodicity

A soil become saline when the concentration of soluble salts increases in the soil profile whereas a soil is considered sodic when the concentration of sodium ions is increased in comparison to calcium and magnesium ions on the soil colloids. Saline soils have good soil aggregation but poor plant establishment due to physiological dryness created by high osmotic pressure due to presence of soluble salts whereas soil is completely dispersed in case of sodic soils due to presence of high concentration of sodium ions which causes dispersion of soil aggregates. There are various factors contributing salinity which includes use of saline water for irrigation purpose, presence of soluble salts in parent material, use of saline soil amendments etc. Sodicity may be caused using poor quality irrigation water and poor water management that cause water logging on the soil surface. Anthropogenic sodicity is called secondary sodification.

11.11.1. Effect of salinity and sodicity

- The three major problems induced by sodicity are poor soil structure, low water infiltration and surface crusting.
- Salinity causes disturbance in nutrient uptake resulting loss in crop yield and quality

11.12. Soil quality Assessment

Soil quality cannot be measured directly because it is a broad, integrative, context-dependent concept. Instead, we analyze a variety of proxy measurements that together provide clues about how the soil is functioning as viewed from one or more soil-use perspectives. These measurements are called soil quality indicators. A set of low-cost readily measured indicators that accurately predict soil functions of interest is called an efficient indicator set. Indicators of soil quality may include characteristics of soil solids, soil solutions, soil atmospheres, vegetation, and other soil biota, and possibly even economic analyses of land-uses or ecosystem services.

lthough the quantity and quality of data may differ, the process of soil quality evaluation follows the same basic steps regardless of the method used: identification of soil use issues followed by indicator selection and interpretation. More specifically, in order to select appropriate indicators, one must first determine the land-use objectives, and then indicators must be proposed, measured and assessed across a representative set of lands and management practices. An efficient indicator set should be used to inform land management decisions at specific sites and then be used to monitor trends in soil function after changing practices and over time.

11.12.1. Why Assess Soil Quality

Soil quality is evaluated to learn about the effects of management practices on soil function. Reasons for evaluating soil quality fall into three categories:

- Awareness and education
- Evaluation of practice effects and trouble-shooting
- Evaluation of alternative practices

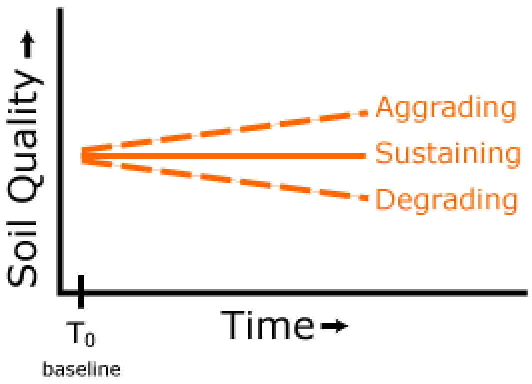
11.13. Awareness and education

The soil quality concept emphasizes an ecological approach to land management. Management actions don't have simple, single effects in complex systems, such as soil. Management has multiple effects, both direct and indirect. For example, tillage is used to loosen surface soil, prepare the seedbed, and control weeds and pests. But tillage can also break up soil structure, speed the decomposition and loss of organic matter, increase the threat of erosion, destroy the habitat of helpful organisms, and cause compaction. Understanding the tradeoffs that exist for the range of management options is a first step towards improved land management and public policy. Assessment as an Educational Tool includes one-on-one and field day use of in-field testing tools.

Evaluation of practice effects and trouble-shooting

Soil quality is often referred to as "Soil Health" because of objectives similar to the monitoring and maintenance of human health. Doctors monitor health indicators and watch for irregularities or declines in status. The set of health indicators measured during a check-up is familiar to all of us: temperature, pulse, blood pressure, heartbeat, urine samples, etc. Monitoring of these indicators may reveal potential problems even before painful symptoms occur; the earlier problems are observed, the easier they are to treat.

Assessment as a monitoring tool:



Dynamic soil quality changes over time

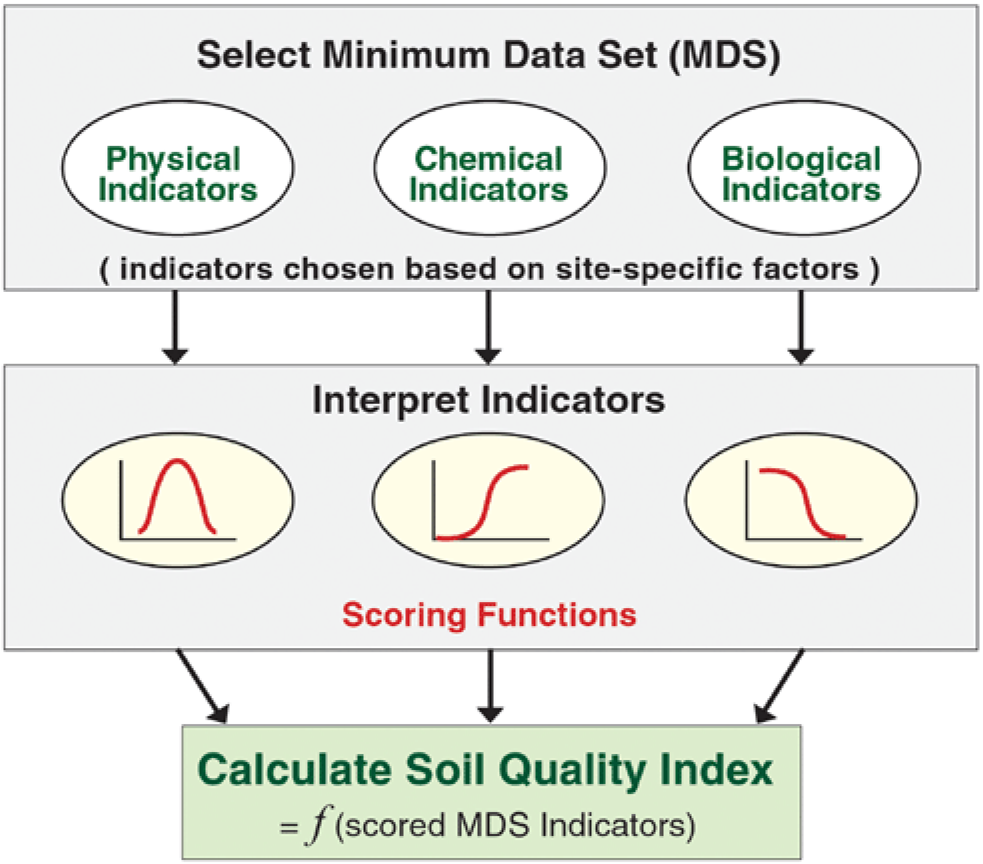
Likewise, soil indicators that appear irregular or decline over time provide a signal that some aspect of the management should be reconsidered. Although soil fertility testing already serves this role in regard to plant nutrition, soil quality assessment expands this to include the wider range of soil functions and environmental outcomes.

11.14. Soil Management Assessment Framework

The Soil Management Assessment Framework (SMAF) provides site-specific interpretations for soil quality indicator results. Because the definition of soil quality for your site depends on your management goals, climate, crops, and soil type, a framework approach to soil quality indexing is used. This allows for the necessary differences in site- and goal-specific interpretations of indicator results. The index framework involves three main steps:

- Indicator selection to efficiently and effectively monitor the critical soil functions
- Interpreting indicators in terms of soil function (using expected ranges determined by the soil's inherent capability)1
- Combining indicator scores into an integrated index of soil quality (optional). The result is a relative measure of the soil's ability to perform the functions necessary for its intended use.

Andrews *et al.*, 2001

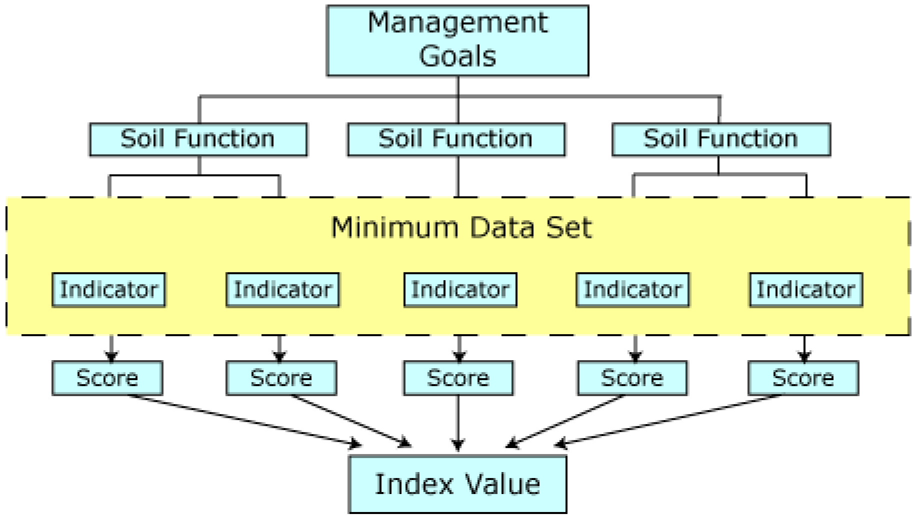


11.15. Indicator Selection

The set of indicators used to determine a soil's quality is also called a minimum data set. To select a minimum data set, two main methods have been established: expert opinion and statistical data reduction.

Expert opinion, by definition, requires expert knowledge of the system. Using a hierarchical framework for choosing the indicators may help make selection more systematic. As shown in the figure below, management goals dictate the soil functions of interest, which in turn, suggest related indicators. For instance, if animal waste disposal is a goal for a particular field, filtering and buffering is an important soil function. Under filtering and buffering, organic matter content and pH are potential indicators.

The indicator set must be further refined according to climate, soil, and plant community or other factors. This is the method used by the Soil Management Assessment Framework.



Statistical data reduction has been demonstration to effectively choose indicators in a number of soil systems (Brejda *et al.*, 2000; Andrews *et al.*, 2001; Andrews and Carroll, 2001). This method can eliminate disciplinary bias that could be a problem with expert selection of indicators but it does assume that appropriate candidate indicators are in the original data set (so a minimum level of knowledge is required). The major weakness of this method is the need for a large existing dataset. It is unlikely that managers will have access to data sets that are suitable in size (either number of indicators measured or number observations made) to make this method feasible for individuals' use

11.16. Combining Indicator Scores

Once scored, indicators can be combined in a variety of ways, such as additive (Andrews and Carroll, 2001), weighted (Karlen et al, 1998), or multiplicative indexes (Doran and Parkin, 1996; Larsen and Pierce, 1991). In a comparison of indexing methods, Andrews et al. (2001) found few differences among index outcomes calculated by differing methods. The Soil Management Assessment Framework index at this site is additive but offers users the option to weight indicator scores.

11.17. General practices for soil health management

- No-tillage
- Cover cropping
- Green manuring

- Addition of organic amendments
- Integrated nutrient management
- Irrigation and drainage
- Cropping system including N-fixing legumes
- Efficient application of fertilizers and nutrients

11.18. References

1. Andrews, S.S. and C.R. Carroll, 2001. Designing a decision tool for sustainable agroecosystem management: Soil quality assessment of a poultry litter management case study. Ecol. Applic. 11 (6): 1573-1585.
2. Andrews, S.S., D.L. Karlen, and J.P. Mitchell. 2001. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agriculture, Ecosystems and Environment 1760: 1-21.
3. Andrews, S.S., D.L. Karlen, and J.P. Mitchell. 2001. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agriculture, Ecosystems and Environment 1760: 1-21.
4. Blum, W. E. (2005). Functions of soil for society and the environment. *Reviews in Environmental Science and Bio/Technology*, *4*(3), 75-79.
5. Brejda, J.J., T.B. Moorman, D.L. Karlen, and T.H. Dao. 2000. Identification of regional soil quality factors and indicators: I. Central and southern high plains. Soil Sci. Soc. Am. J. 64: 2115–2124.
6. Brejda, J.J., T.B. Moorman, J.L. Smith, D.L. Karlen, D.L. Allan, and T.H. Dao. 2000. Distribution and variability of surface soil properties at a regional scale. Soil Sci. Soc. Am. J. 64:974–982.
7. Doran, J. W., and Parkin, T. B. (1994). Defining and assessing soil quality. *Defining soil quality for a sustainable environment*, (definingsoilqua), 1-21.
8. Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H.A. Shayler, M.B. McBride, K.S.M Kurtz, D.W. Wolfe, and G.S. Abawi, (2016) Comprehensive Assessment of Soil Health – The Cornell Framework, Edition **3.2**, Cornell University, Geneva, NY.

Chapter- 12

Deficit irrigation strategies for improving water productivity and management of abiotic stress in horticulture crops

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12.1 Introduction

Water is also becoming scarce not only in arid and drought prone areas but also in regions where rainfall is abundant. Also, due to climate changes and increased demands of different water users (agriculture, industry, domestic) water becomes scarce resources worldwide. Since, irrigated agriculture is the one of the largest consumer of these resources (so-called blue water footprint), irrigation management must be shifted from maximal production per crop area to maximal production per unit of water used by crops. To cope with the water shortage, it is necessary to adopt water-saving agriculture counter measures. Efficient use of water by irrigation is becoming increasingly important. Among the strategies for reducing water footprints, changing the full irrigation to the reduced crop's water supply (deficit irrigation techniques) is one of the options. In recent years, water-saving irrigations techniques such as deficit irrigation (DI) and partial root zone drying (PRD) or alternative irrigation (AI) have been developed with micro irrigation system for field and horticulture crops. These systems improve the water productivity (WP) and quality of produce in horticulture crops as well as in cereal crops.

12.2. Water efficient technologies/strategies

12.2.1. Deficit irrigation (DI)

Deficit irrigation is an optimization strategy in which irrigation is applied during drought-sensitive growth stages of a crop. The correct application of DI requires thorough understanding of the yield response to water (crop sensitivity to drought stress). In regions where water resources are restrictive it can be more profitable for a farmer to maximize crop water productivity instead of maximizing the harvest per unit land.

12.2.2. Concept of deficit irrigation

Water restriction is limited to drought-tolerant phenological stages, often the vegetative stages and the late ripening period. Total irrigation application is therefore not proportional to irrigation requirements throughout the crop cycle. While this inevitably results in plant drought stress and consequently in production loss, DI maximizes water productivity, which is the main limiting factor (English, 1990). In other words, DI aims at stabilizing yields and at obtaining maximum WP rather than maximum yields (Zhang and Oweis, 1999).

In the literature, the terms ‘supplemental irrigation’ and ‘deficit irrigation’ are both used. The first term generally refers to a rain-fed crop receiving additional irrigation during the whole season or during sensitive growth stages, whereas DI generally refers to fully irrigated crops from which water is withheld during certain tolerant growth stages.

Since drought tolerance varies considerably by genotype and by phenological stage, DI requires precise knowledge of crop response to drought stress for each of the growth stages (Kirda et al., 1999). In addition, correct application of DI requires a thorough assessment of the economic impact of the yield reduction caused by drought stress (English, 1990; English and Raja, 1996; Sepaskhah and Akbari, 2005; Sepaskhah et al., 2006). In areas where water is the most limiting factor, maximizing WP may be economically more profitable for the farmer than maximizing yields (English, 1990). As these examples suggest, DI requires a highly integrated approach to agricultural water policy.

12.2.3. Advantages

- Maximizes the water productivity.
- Although a certain reduction in yield is observed but the quality of the yield (e.g. sugar content, grain size) observed to be equal or even superior to rain-fed or FI cultivation
- Allows economic planning and stable income due to a stabilization of the harvest in comparison with rainfed cultivation
- Decreases the risk of certain diseases linked to high humidity (e.g. fungi) in comparison with full irrigation
- Reducing irrigation applications over the crop cycle will also reduce nutrient loss through leaching from the root zone, resulting in improved ground water quality
- Over-fertilization may cause crops to be more susceptible to dry spells and may lead to decreased harvest indexes.
- Lower fertilizers needs as compared to in full irrigation. DI reduced fertilizer application. Combining DI and optimum fertilizer application leads to a higher yield

increase (higher WP) than the sum of the separate yield increases obtained by both factors

- Controls of vegetative growth and canopy density (reduce pruning in grapevine)
- Improvement of irrigation water use efficiency and saving water for irrigation
- Increases in nutrient use efficiency (especially N)
- Improvement of fruit or yield quality (potato, grape, tomato, pepper, apple, maize)
- DI is the possibility of controlling sowing dates by irrigation, which allows improved planning of agricultural practices
- Due to drought stress in particular growth stages, the length of the cropping cycle might change under rain-fed cultivation. Farre´ and Faci (2006) report a delay in flowering (7 and 17 days) and maturity (5 and 12 days) for sorghum and maize, respectively, under water deficit conditions. McMaster and Wilhelm (2003) find that drought decreases crop cycle length for wheat and barley.

12.2.4.Constraints

- Exact knowledge of the crop response to water stress is important.
- There should be sufficient flexibility in access to water during periods of high demand (drought sensitive stages of a crop).
- A minimum quantity of water should be guaranteed for the crop, below which DI has no significant beneficial effect.
- An individual farmer should consider the benefit for the total water users community (extra land can be irrigated with the saved water), when he faces a below-maximum yield
- Because irrigation is applied more efficiently, the risk for soil salinzation is higher under DI as compared to full irrigation.
- Determining optimal timing of irrigation applications is particularly difficult for crops with CWP functions in which maximal WP is found within a small optimum range of ET
- Irrigators should have unrestricted access to irrigation water during sensitive growth stages.

The effect of water stress on plants at physiological, biochemical and molecular levels and a crop that is imposed to PRD as a water-saving irrigation may show diverse responses to water stress in terms of these three responses levels according to the severity and timing of the water stress (Fig 1).

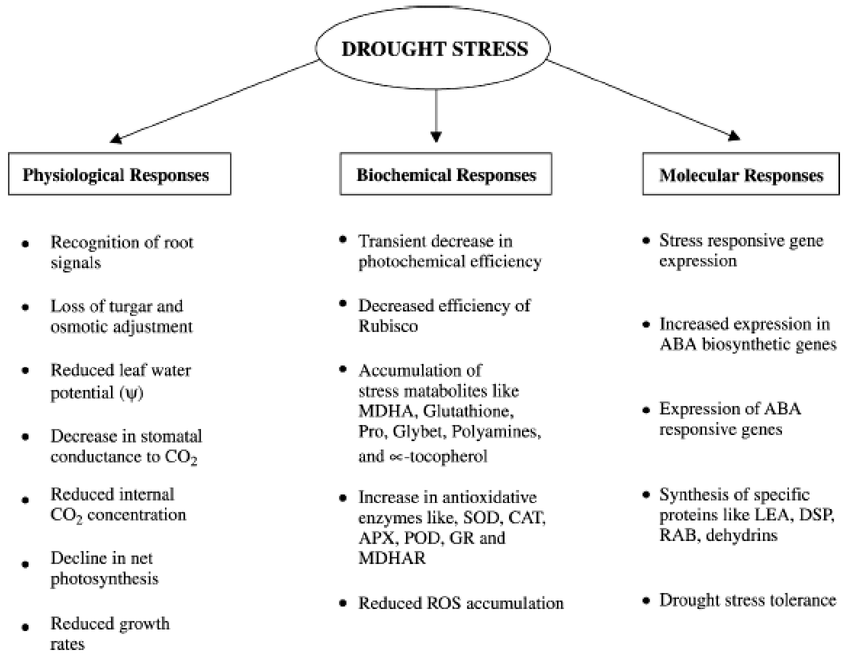


Fig 1. Physiological and molecular bases of drought stress tolerance (After Shao et al., 2008).

12.3. Strategies to improve water productivity under water scarcity

There are two ways to improve water productivity of crop**:**

- Cultivation of plants with high water-use efficiency or plants with greater drought tolerance
- 2. Investment in water-efficient technologies for growing plants as in deficit irrigation techniques

In recent years, the two main approaches for developing practical solutions to manipulate vegetative and reproductive growth used. That has been: Regulated deficit irrigation (RDI) and Partial root zone drying (PRD).

12.3.1. RDI

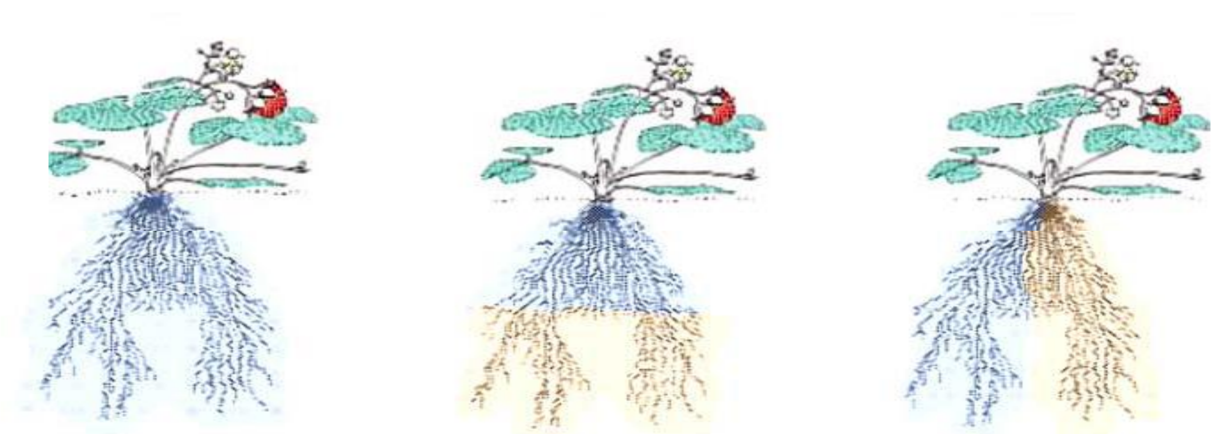
- irrigating at less than the full requirement of the plants and potential evapotranspiration (maintaining soil moisture at a relatively low level).
- imposes **plant deficit** during prescribed crop growth period

12.3.2.PRD

- applying irrigation to alternately wet and dry (at least) two spatially prescribed parts of the plant root system to simultaneously maintain plant water status at maximum water potential and control vegetative growth for prescribed parts of the seasonal cycle of plant development
- imposes **soil deficit** within alternating sides of rootzone but plants remains turgid

RDI and PRD have become established water management techniques. Therefore, great emphasis is placed in the area of crop physiology and crop management with the aim to make plants more efficient in water use through RDI and PRD irrigation practice under dry conditions. **Partial root-zone drying irrigation (PRD)**

Partial root-zone drying (PRD) is a modified form of deficit irrigation (DI) (English et al., 1990), which involves irrigating only one part of the root zone in each irrigation event, leaving another part to dry to certain soil water content before rewetting by shifting irrigation to the dry side; therefore, PRD is a novel irrigation strategy since half of the roots is placed in drying soil and the other half is growing in irrigated soil (Ahmadi et al., 2010a).



Full irrigation **Deficit irrigation** **PRD**

Fig 2 : Schematic of the irrigation pattern in FI, DI, and PRD (After Davies and Hartung, 2004).

12.3.3. Principle of PRD

When a part of the root zone dries out, ABA produced in the roots in drying soils and is transported by water flow in xylem to the shoot for regulating the shoot physiology. The increase in abscisic acid in the xylem flow roots to leaves triggers the closure of stomata as response to water stress and reduced shoot growth and transpiration. After 10–15 days, the wet and the dry root zone are inverted. However, due to alternating wet and dry zones, roots have continuous access to water. Thus, the plant continues to grow and flowering and fruit development will not affect. Alternating the wet and dry zones of the roots means that repeated surges of ABA are delivered to the shoots, maintaining conditions of reduced shoot growth and reduced transpiration, but with no significant effects on flowering and fruit development (Fig 3)

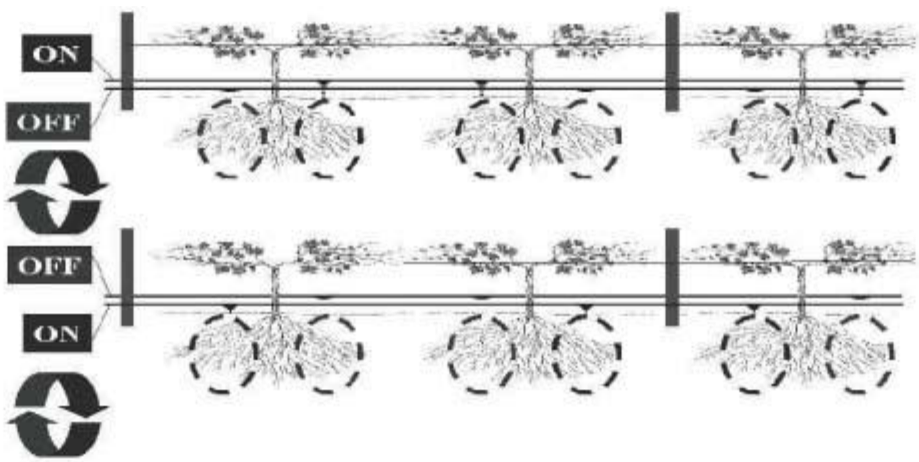


Fig 3 : Partial root zone drying using two above-ground drip lines in a vineyard

12.3.4. Chemical and hydraulic signaling in PRD

In drought, soil drying induces restriction of water supply and these results in a sequential reduction of tissue water content, growth and stomatal conductance. The plants have some mechanism for sensing the availability of water in the soil and regulating stomatal conductance and leaf growth accordingly. It has been termed non-hydraulic or chemical signaling. Hydraulic signaling, which represents transmission of reduced soil water availability *via* changes in the xylem sap tension. Roots in drying soil produce more ABA than under normal conditions and it is moved as an anti-stress root chemical signal to shoot through transpiration stream and limits the stomatal conductance. At mild water stress, ABA as a major chemical signal (CS) acts earlier than the change in plant water status i.e hydraulic signal, HS. However, under severe water stress, both CS and HS may be involved in regulating plant physiological processes. At severe water stress, the leaf water potential in mesophyll cells decreases and stomata will close to a greater extent that inhibits the photosynthetic rate (Taiz and Zeiger, 2006). In some plants, CS and HS occur independent of each other, while in others they take place dependently. A balance between CS and HS occur in PRD. In PRD, roots on the irrigated side absorb enough water to maintain high shoot water potential, and the roots on the non-irrigated side produce ABA for possible reduction in stomatal conductance. This mechanism optimizes water use and increase water productivity.

Difference between RDI and PRD

RDI	**PRD**
Site must be responsive to irrigation	
Can be used with furrow irrigation	Drip irrigation preferred, alternate row furrow possible
Water must be available on demand	
Control of fruit size	No/ negligible effect on size
Vegetative growth control	Vegetative growth control
Potential for yield loss	No loss of yield
Positive effects on fruit quality	Possible improvement in quality
Marginal water savings	Significant water savings
No irrigation hardware modification	Significant changes required. Can be retrofitted.
Soil water monitoring recommended	
High-level management skills required	

Source: Regulated deficit irrigation and Partial rootzone drying, Irrigation insights no 4

12.3.5. Agricultural benefit of root-to-shoot chemical signaling

PRD reduced vine vigour, canopy density and increased the quality, yield of fruit and improved water-use efficiency (Loveys et al., 2000). It also resulted in leaf expansion rate in wheat (Ali et al., 1998), maize (Bahrun et al., 2002), soybean (Liu et al., 2005a), potato (Liu et al., 2006c), and tomato (Topcu et al., 2007). Excessive plant vigour is a major problem for many fruit crops, since the use of assimilates in leaf growth restricts fruit set and development.

The frequency of the switch is determined according to soil type and other factors such as rainfall and temperature. In most of the published data the PRD cycle includes 10 to 15 days (Davies et al., 2000; Stoll et al., 2000).

12.3.6. Advantages and disadvantages of PRD irrigation

PRD irrigation may have benefits on water use, WUE, fruit quality and nutrient uptake

It is important to assess how much water PRD can save in a growing season. Water-saving considerations have resulted in most PRD treatments receiving less water (usually 50%) than control plants. In addition to water savings, PRD has also been reported to have beneficial effects on fruit quality and nutrient uptake with no, or minimal, losses in yield (dos Santos *et al.*, 2003).

12.3.7. Water use and WUE

Water use as percent of fully irrigated treatment is decreased and irrigation water use efficiency (IWUE) is increased essentially by PRD as reported in a number of species, e.g. cotton, tomato, pear grapevine and hot pepper (Table1). In maize PRD irrigation reduced water consumption by 35% with a total biomass reduction of 6–11% as compared with fully watered plants (Kang and Zhang, 2004). Another experiment with hot peppers and drip irrigation showed that PRD reduced water used for irrigation by about 40% and maintained similar yield as in fully watered plants (Kang et al., 2001).

PRD was tested in peach and apple orchards at Yangling, Shaanxi, China by using a drip irrigation system (Gong *et al.*, 2001), and in a pear orchard in Victoria, Australia by using a flood irrigation system (Kang *et al.*, 2002**b**). Results showed water savings of 52% in peach and 23% in pear, respectively **(Kang and Zhang, 2004**).

12.3.8. Fruit quality

PRD can improve the quality of fruits of several species; in grapes, cotton, tomato, and hot pepper (Table 1). In grapes sugar content was increased by PRD (e.g. Stoll et al., 2000, dos Santos *et al.*, 2003). They have shown that this is largely a result of better control of vegetative growth of the grapevine. Also Dry et al., (2000) found that wine quality was consistently higher from PRD Wine yards.

Table1. Effect of PRD on water use, WUE and fruit quality

Plant	Water use as % of fully irrigation	Fruit Quality	IWUE as % of fully irrigation	Reference
cotton	70	I*	134	Tang et al.,(2005)
Tomato	50	I	163	Kirda et al (2004)
Pear	55	n.m	145	Kang and Zhang(2004)
Grapevine	50	I	152	Dry et al(2000)
Hot Pepper	50	I	166	Dorji et al. (2004)

*I= improvement of quality n.m= not measured

12.3.9. Nutrient uptake

An extra benefit from PRD-induced new roots may be related to their function in nutrient uptake. The drying and rewetting cycle by PRD induced new roots, and this may make the nutrients in soil zone more available to the plants (Kang et al., 2001, dos Santos *et al.*, 2003).

12.3.10. Root development and water uptake

Root development and distribution are affected by spatial and temporal soil water distribution (Wang et al., 2006). Further, they affect water and nutrient uptake from the soil to maintain the physiological activities of the above-ground part of the crop. Mild water stress in soil leads to preferential root growth into the moist soil zone and water uptake through root system expansion and increasing root length density (RLD, cm root per cm3 soil) (Benjamin and Nielsen, 2006; Songsri et al., 2008). Earlier studies indicated that PRD enhanced the extension and inhibition of primary and secondary roots (Kang et al., 2000b), increased root growth (Dry et al., 2000) and root mass (Kang et al., 2000a; Mingo et al., 2004), improve ABA-induced root hydraulic conductivity (Glinka, 1980; Taiz and Zeiger, 2006; Thompson et al., 2007), and increased the nutrient uptake (Wang et al., 2009).

Plant water uptake rate is enhanced after re-watering in water stress condition compared to full irrigation. This is obtained due to improvement of hydraulic conductivity of root systems that is subjected to water stress (Kang and Zhang, 2004). The root system can partially compensate for the increasing limited water availability on the non-irrigated side of PRD due to an increase in root hydraulic conductivity.

12.3.11. Disadvantages of PRD irrigation

PRD may be reducing biomass production as CO_2 uptake is partly restricted due to stomatal closure causing water savings. Biomass reductions are often in the range of 10% in cereal crops, while in fruit trees hardly any yield reduction has been found.

The value of benefits from water savings should be balanced with value of yield reductions and cost of implementing PRD irrigation system compared with traditional systems. As PRD irrigation is in the research phase further experiences are needed to evaluate economical advantages of PRD irrigation.

12.3.12. Practical application of RDI and PRD: Irrigation management strategies

Before making irrigation plan it is important to know the characteristics of soil in the field including:

- Number and thickness of layers (identifying impermeable layers in the soil that may cause drainage and surface run-off problems)
- Soil texture,
- Soil structure
- Field water capacity, wilting point
- Rate of infiltration
- Rooting depth of plants that will be growing

• Soil chemical analyses to identify possible chemical/nutrient problems (e.g. acidity, salinity, nutrient deficiency).

12.4. Irrigation methods for applying RDI and PRD

PRD and RDI could be applied in the field by different irrigation methods including:

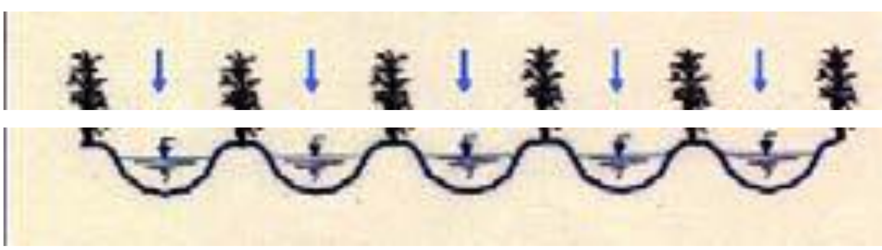
• Furrow irrigation

• Drip irrigation

12.4.1. Furrow irrigation system

PRD System should be applied as the two rows configurations and the both furrows should be irrigated alternately. After the switching period, wetted furrow started to dry out and dry furrow will be irrigated.

RDI System should be applied at the same time in all rows, but with 50-70% water amount needed for full treatment



In drip surface or subsurface for PRD irrigation two irrigation lines should be set up and operated separately with the distance between emitters of 60cm (for potato). This way lateral of one emitter will irrigate one part of the root system and emitters of other lateral will irrigate other half of root system. In FI and RDI irrigation one lateral is used for irrigation with the distance of 30 cm between emitters. Irrigation in FI and RDI should cover a total root area.

12.4.2. Precaution to be taken while implementing PRD

- Best PRD responses occur in soils with high values of readily available water (RAW). Shallow soils with low RAW can allow relatively small volumes of applied water to deplete rapidly. To some extent this can be overcome by more frequent irrigation.
- Use of PRD in soils with poor infiltration characteristics may also cause problems if sufficient water cannot be supplied through what is effectively 50% of the normal soil surface area.
- The amount and timing of irrigation applied to the 'wet' side should be sufficient to prevent the development of significant water deficits (soil moisture tension should remain higher than 50 kPa).
- If soil moisture monitoring is available, the irrigated side of the plant should be switched when water extraction from the "dry" side becomes negligible. In sandy soils and under hot dry conditions this may be only a few days. In soils with a higher water retention characteristic and under less stressful conditions, the cycle time may become several weeks.
- Use of PRD should not result in significant reduction in midday leaf water potential when compared with standard irrigation practice.
- When PRD is being implemented in an existing orchard, total soil area wetted by the irrigation system (wet plus dry sides) should not vary significantly from that wetted by the original irrigation system. For example, conversion from flood to drip may wet only a small fraction of the available roots. The PRD irrigation system should aim to wet about half the roots at any one time.
- Correctly implemented PRD should not result in major effects on fruit quality. With Navel oranges, PRD using very low water application rates saw a reduction in fruit size in heavily cropped trees but this problem was not evident at higher water inputs. A reduction in water input, applied by flood or by drip, may result in a small but significant reduction in the percentage of juice and an increase in acid. There should be no effect on sugars and sugar/acid ratios may change accordingly.
- Response to PRD varies between species. It is still not known how some plants will respond.

12.5. Conclusion

In areas where the available water supply limits agricultural production, deficit irrigation will gain importance over time as farmers strive to increase the productivity of their limited land and water resources. Farmers must choose crops and irrigation strategies carefully to maximize the value of their crop and livestock production activities, while ensuring the sustainability of agriculture. Deficit irrigation will play an important role in farm-level water management strategies, with consequent increases in the output generated per unit of water used in agriculture.

Partial root zone drying strategy is a very useful and significant step in improving the water use efficiency, increasing productivity, and improving quality of produce of perennial horticultural crops. The cost of implementing PRD is economical where the cost of irrigation water is high and as water becomes an increasingly valuable and scarce resource.

12.6. References:

1. Ahmadi, S.H., Andersen, M.N., Plauborg, F., Poulsen, R.T., and Hansen, S., 2009. A quantitative approach to developing more mechanistic gas exchange models for field grown potato: A new insight into chemical and hydraulic signalling. Agri. and Forest Meteorology, 149: 1541-1551.
2. Ahmadi, S.H., Andersen, M.N., Plauborg, F., Poulsen, R.T., Jensen, C.R., Sepaskhah, A.R., Hansen, S., 2010a. Effects of irrigation strategies and soils on field grown potatoes: Gas xchange and xylem [ABA]. Agri. Water Management, 97: 1486-1494.
3. Benjamin, J.G., Nielsen, D.C., 2006. Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Research, 97: 248-253.
4. Davies, W.J., Zhang, J.H., 1991. Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology, 42: 55-76.
5. Dos Santos TP, Lopes CM, Rodrigues ML, de Souza CR, Maroco JP, Pereira JS, Silva JR, Chaves MM. 2003. Partial rootzone drying: effects on fruit growth and quality of field grown grapevines (*Vitis vinifera*). Funct. Plant Biol. 30, 663–671.
6. Dry, P.R., Loveys, B.R., During, H., 2000. Partial drying of the rootzone of grape. II. Changes in the pattern of root development. Vitis, 39: 9-12.
7. Dry PR, Loveys BR**.** 2000. Partial drying of the rootzone of grape. I. Transient changes in shoot growth and gas exchange. *Vitis* 39, 3–7.
8. Dorji K, Behboudian MH and Zegbe-Dominguez JA (2005) Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial root zone drying. Scientia Horti. 104: 137-149.

9. English, M.J, Musick, J.T., Murty, V.V.N., 1990. Deficit irrigation. In: Management of farm irrigation systems (Hoffman, G.J., Howell, T.A., and Solomon, K.H., Editors). ASAE onograph no. 9. American Society of Agricultural Engineers publisher, 1020p.
10. Kang, S.Z., Hu, X., Goodwin, I., Jerie, P., 2002. Soil water distribution, water use, and yield response to partial root zone drying under a shallow groundwater table condition in a pear orchard. Scientia Horticulturae, 92: 277-291.
11. Kang, S.Z., Hu, X., Jerie, P., Zhang, J.H., 2003. The effects of partial rootzone drying on root, trunk sap flow and water balance in an irrigated pear (*Pyrus communis* L.) orchard. Jour. of Hydrology, 280: 192-206.
12. Kang SZ, Zhang. J. 2004. Controlled alternate partial root-zone irrigation: its physiological consequences and impact on water use efficiency. J. Exp. Bot. 55, 407, 2437–2446
13. Kirda, C. Cetin, M., Dasgan,Y., Topcu, S., Kaman, H., Ekici, B., Derici, M.R. & Ozguven A.I.(2004)Yield response of greenhouse grown tomato to partial root drying and conventional deficit irrigation. Agricultural Water Management 69. 191–201
14. Liu, F., Shahnazari, A., Andersen, M.N., Jacobsen, S.E., Jensen, C.R., 2006. Effects of deficit irrigation (DI) and partial root drying (PRD) on gas exchange, biomass partitioning, and water use efficiency in potato. Scientia Horticulturae, 109: 113-117.
15. Liu, F., Song, R., Zhang, X., Shahnazari, A., Andersen, M.N., Plauborg, F., Jacobsen, S.E. Jensen, C.R., 2008. Measurement and modeling of ABA signaling in potato (*Solanum tuberosum* L.) during partial root-zone drying. Environ. and Experimental Botany, 63: 385-391.
16. Liu, F., Andersen, M.N., Jensen, C.R., 2009. Capability of the 'Ball-Berry' model for predicting stomatal conductance and water use efficiency of potato leaves under different irrigation regimes. Scientia Horticulturae, 122: 346-354.
17. Mingo, D.M., Theobald, J., Bacon, M.A., Davies, W.J., Dodd, I.C., 2004. Biomass allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone drying: enhancement of root growth. Functional Plant Biology, 31: 971-978.
18. Paul E. Kriedemann and Ian Goodwin. Manual on Regulated deficit irrigation and partial rootzone drying. Irrigation Insights No. 4. Published by Land and Water, Austrelia. Pp:179

19. Rodrigues, M.L., Santos, T.P., Rodrigues, A.P., de Souza, C.R., Lopes, C.M., Maroco, J.P., Pereira, J.S., Chaves, M.M., 2008. Hydraulic and chemical signalling in the regulation of stomatal conductance and plant water use in field grapevines growing under deficit irrigation. Functional Plant Biology, 35: 565-579.
20. Sadras, V.O., 2009. Does partial root-zone drying improve irrigation water productivity in the field? A metaanalysis. Irrigation Science, 27: 183-190.
21. Sam Geerts and Dirk Raes 2009 Review: Deficit irrigation as an on-farm strategy to maximize crop water productivity.in dry areas Agricultural Water Management 96: 1275–1284
22. Shahnazari, A., Ahmadi, S.H., Lærke, P.E., Liu, F., Plauborg, F., Jacobsen, S.E., Jensen, C.R., Andersen, M.N., 2008. Nitrogen dynamics in the soil-plant system under deficit and partial root-zone drying irrigation strategies in potatoes. Europ. Jour. of Agro., 28: 65-73.
23. Shani-Dashtgol, A., Jaafari, S., Abbasi, N., Malaki, A., 2006. Effect of alternate furrow irrigation (PRD) on yield quantity and quality of sugarcane in southern farm in Ahvaz. Proceeding of national conference on Irrigation and Drainage Networks Management. Shahid Chamran University of Ahvaz. 2-4 May, Pp: 565-572.
24. Sepaskhah A.R. and S.H. Ahmadi. 2010. A review of Partial root zone drying irrigation. International Journal of Plant Production (2010) 4(4): 241-258
25. Stoll M, Loveys B, Dry P. 2000. Improving water use efficiency of irrigated horticultural crops. J. Exp. Bot. 51, 1627–1634.
26. Tardieu, F., Davies, W.J., 1993. Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. Plant, Cell and Environment, 16: 341-349.
27. Thompson, A.J., Andrews, J., Mulholland, B.J., McKee, J.M.T., Hilton, H.W., Horridge, J.S., Farquhar, G.D., Smeeton, R.C., Smillie, I.R.A., Black, C.R., Taylor, I.B., 2007. Overproduction of Abscisic acid in tomato increases transpiration efficicency and root hydraulic conductivity and influences leaf expansion. Plant Physiology, 143: 1905-1917.
28. Wahbi, S., Wakrim, R., Aganchich, B., Tahi, H., Serraj, R., 2005. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate I. Physiological and agronomic responses. Agriculture, Ecosystems and Environment, 106: 289-301.

29. Wang, F.X., Kang, Y., Liu, S.P., 2006. Effects of drip irrigation frequency on soil wetting pattern and potato growth in North China Plain. Agri. Water Management, 79: 248-264.

30. Zegbe, J.A., Behboudian, M.H., 2008. Plant water status, CO2 assimilation, yield, and fruit quality of 'Pacific RoseTM' apple under partial rootzone drying. Advances in Horti. Sci., 22: 27-32.

31. Zegbe, J.A., Behboudian, M.H., Clothier, B.E., 2004. Partial rootzone drying is a feasible option for irrigating processing tomatoes. Agri. Water Management, 68: 195-206.

32. Zhang, H., 2003. Improving water productivity through deficit irrigation: Examples from Syria, the north China Plain and Oregon, USA. In: Water Productivity in Agriculture: Limits and Opportunities for Improvement (Kijne, J.W., Barker, R., and Molden, D. eds). CABI publishing, 332p.

Chapter- 13

Climate- smart agriculture Abiotic stress effect on horticulture crop yield and quality

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Climate Smart Agriculture (CSA) is defined as Agricultural practices that help to guide actions required to modify & reorient effectively support the development & guarantee food security during ever- changing climate. Climate change is a problem with the highest priority facing the farming community today, influencing horticultural production nationwide.

13.1. Crop yield & climate

Plant physiology has been greatly influenced by climate variability by several means environmental extremes & climate variability enhanced the chances of numerous stresses on plants. Climate change affects crop production by means of direct, indirect & socio-economic effects as described in fig.1

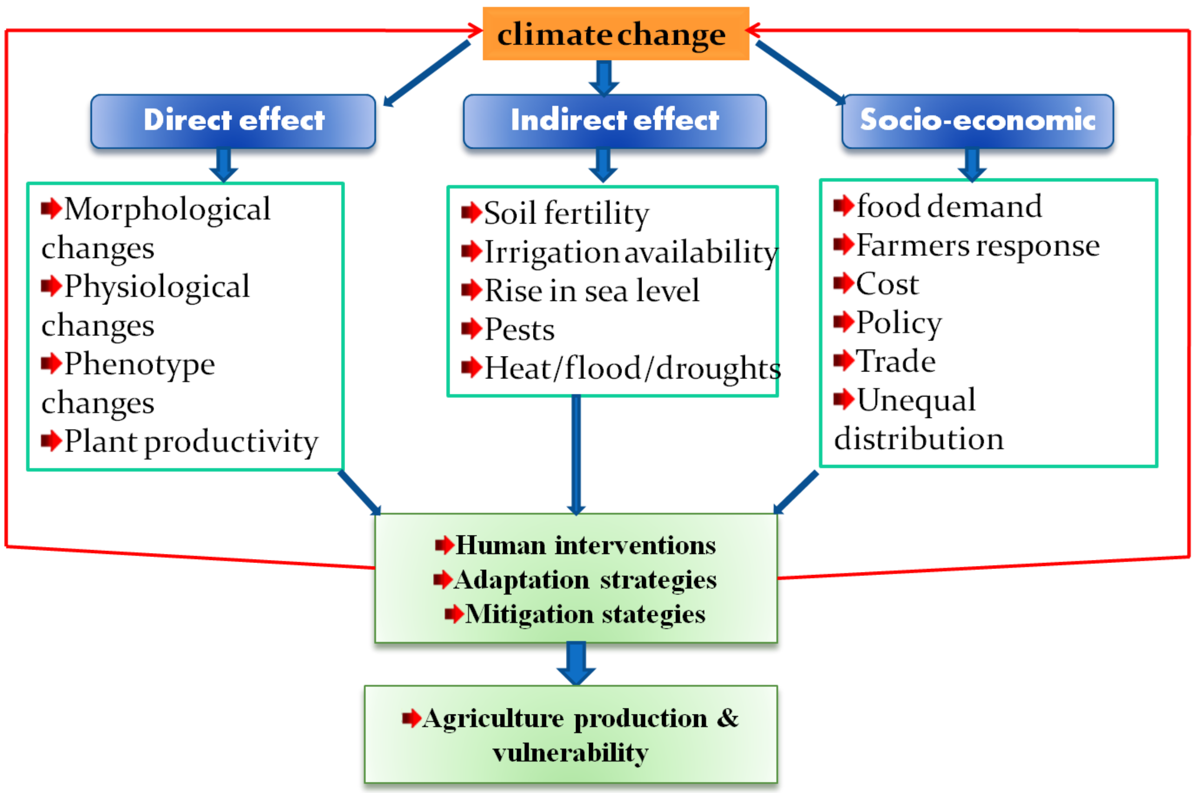


Fig.1 Direct, indirect, & socio-economic effects of climate change on Agril. Production

13.2. Crop Adaptation & over all climate stresses

With the increase of the earth's temperature, the climate undergoes severe alterations & becomes abiotically stressful. Environmental changes are very damaging & pose various threats to naturally prevailing crop species. Under field conditions, drought & heat are the most pre dominant stresses & have a significant influence on plants. It is reported that plants require an optimum temperature for their normal growth & blooming. Plant physiology is heavily influenced by temperature fluctuations. As heat stress affects the grain production & yield, cold stress results in impaired fertility & drought stress negatively influences the morpho-physiology of plants.

Overall, global warning & climate change both have some negative & positive effects on agricultural crops, as explained in fig,. 1.

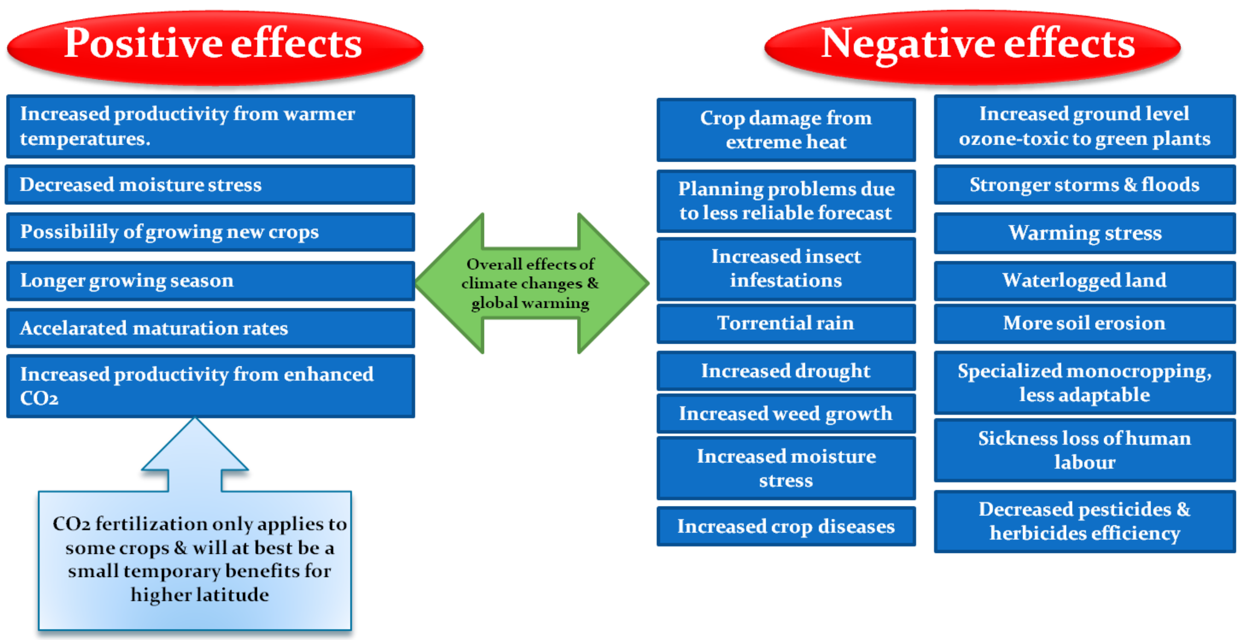
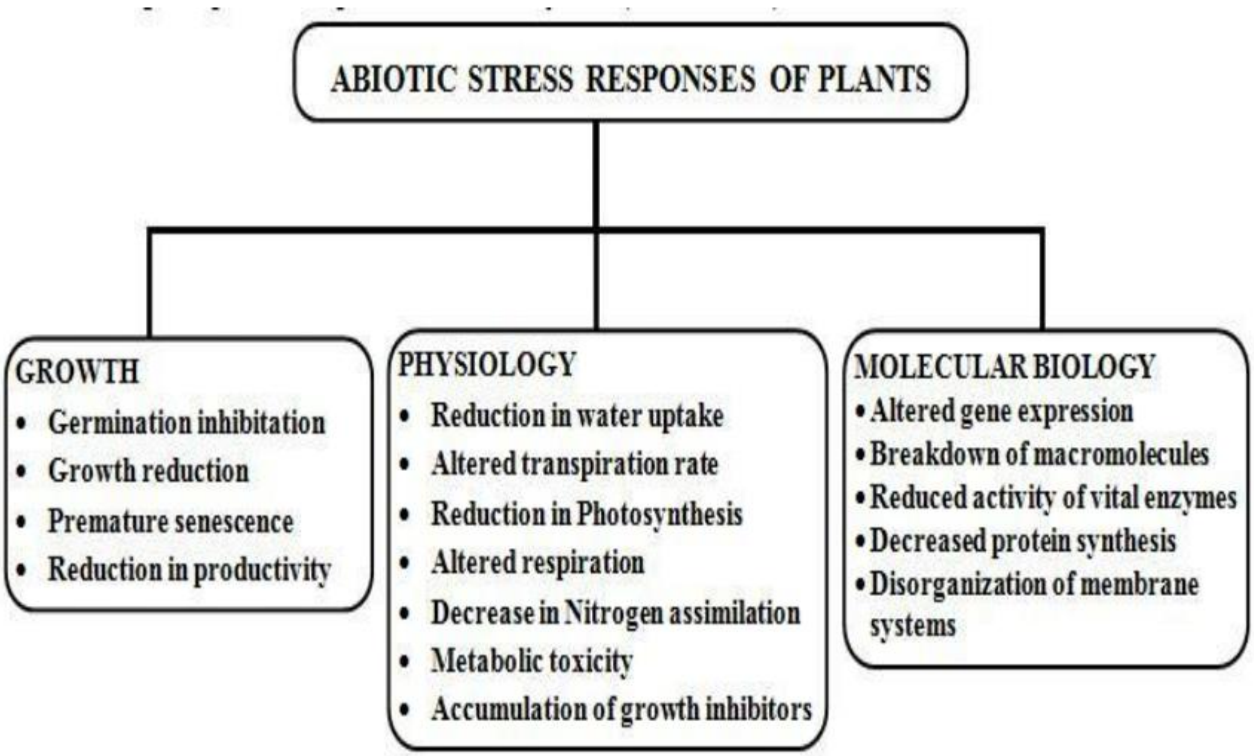


Fig. 2. Overall positive & negative effects of climate change & global warming on crops.

In this context, understanding the stress resistance processes in plants has emerged as a very difficult task for plant scientists in order to develop stress resistance plants. Therefore, sustaining crop yield is an important task in current agriculture & to produce stress tolerant crop plants.



13.3. Physiological effects due to high temperature

Inhibition of metabolic activities due to emergence delay, Simple proteins are less susceptible than complex ones, Changes in membranes fluidity, Respiration rate is doubled for every 0 degree Celsius, increase in tissue temperature.

13.4. Effect of low temperature stress on crop productivity

Flowering is extremely sensitive in low temperature, Wilting of leaves, Browning, Leaf necrosis leading to plant death

13.5. Drought stress on crop productivity

Physiological responses, Recognition of roots signals, Loss of turgar & osmotic adjustment, Decline in net photosynthesis, reduced root shoot growth rates, Biochemical responses, Reduced ROS accumulation

13.7. Biostimulant used in stress mitigation

Use of humic substances, Sea weed extracts, Protein hydrolysates & amino acids etc.

13.8. Mode of application of Biostimulant (Through soil & foliar sprays)

Effects of Biostimulant use in Growth responses, Improved root and shoot growth, Higher flowering & fruit set, Better yield & quality, Biotic stress resistance, Resistance to fungal, bacterial & viral pathogens, Raesistance to insect pests

13.9. Abiotic stress resistance

Salt and drought resistance, freezing & chilling resistance, Enhanced photosynthesis, Nutrient uptake for Enhanced nutritional efficiency.

To summarise, Abiotic stress management as a long term approach involves in understanding physiological mechanism & genetic improvement. There is a great scope for research & development for use of biostimulants as plant Abiotic stress management in India to avoid losses caused due to climate change & help horticultural farming community to improve their crop yield & quality for better returns of their farm income. This will be of great path for fulfilling for research scientists to accept challenges for better opportunities in climate change Agriculture for higher horticulture production in India.

Chapter- 14

An Overview of Grape Cultivation in India under Climate Change Scenario

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14.1. Abstract

Grape (*Vitis vinifera* L.) is a widely consumed fruit in fresh as well as in processed forms viz., raisins, wine, juice, etc. Basically grape is a temperate origin crop but with alterations in production technology it has been successfully adopted to tropical conditions in India. It is supposed to be highly sensitive towards the environment and simultaneously carry highly manipulated canopy architecture. Thereby, the production technology of grape is a unique expression of science and art for better economics to ultimate beneficiary, the farmer. From the researchers' point of view, grape cultivation is like carving of precious stones in spite of many models already has been sculptured. Though as on date India stands the first in productivity, under changing climatic conditions there is lot of scope for research on mitigation of abiotic stresses to achieve the goal of 'doubling farmers' income'. For designing an experiment for these studies, thorough knowledge of production technology of grape is a prerequisite. Therefore, efforts have been made to compile information under the heading 'An Overview of Grape Cultivation in India', with the aim of opening a flood gate of discussions for micro-research projects in viticulture under various abiotic stresses and horticulture in broader sense.

Keywords: grape, viticulture, production cycle, physiological disorder, canopy management

14.2. Introduction

Grape (*Vitis vinifera* L.) is an important fruit crop in India. Basically temperate crop is adapted for tropical parts of the country with modifications in viticulture practices. Grape has its ancient origin during Neolithic age (6500BC to 4000BC) at Armenia and North America. In India mentions of grape are found during Indus Valley Civilization (4000BC), but the modern grape cultivation dates to 1300AD that entered in Maharashtra during 1430AD. The commercial cultivation of grapes started in Maharashtra from 1950AD onwards.

14.2.1. Scope for grapes in abiotic stress research

- High profile crop- consumption, growing, research
- Crazy products and quality attributes
- Wide adaptability to soil and climatic conditions
- Sensitivity towards environmental factors

- Early response to the inputs
- Highly manipulated & unique production technology
- Accumulation of secondary metabolites during stress
- *Terroir* effect- the geographical identity of products
- Possibility of technology applications in other crops

14.2.2. Importance of grapes and its health benefits:

- Grape have been consumed in various forms i.e. fresh grapes, raisins, juice, wine, and other processed forms
- It helps to cure asthma and migraine
- Effective remedy against breast cancer
- Prevents heart attacks and lowers cholesterol levels
- Strengthens bones and prevent onset of osteoporosis
- Provide relief from constipation and indigestion
- Boost immune system and prevent fatigue
- Reduce risk of kidney diseases
- Prevents loss of vision and macular degeneration
- Improve brain function and prevent Alzheimer's disease

14.3. Area, distribution and production

Grapes are originally from temperate habitat and the grape growing area is distributed in between 30 to 50 degree longitude both in north and south hemisphere. However, with modifications in cultivation practices it is successfully being grown under tropical conditions too. Out of 180 grape producing countries India ranks 16th in terms of area under grapes and 7th with respect to production. India thus ranks the first in grape yield per unit area with average production of 21.23 MT/ha. Grape production in India is mainly for fresh grape consumption and raisins. In India Maharashtra makes the largest share in grape production (79.08%).

14.3.1. Grape cultivars

- More than 10000 cultivars - vast majority belong to *Vitis vinifera*

- Cultivar classification on the basis of
    - viticultural characters (maturity, vigour, productivity),
    - phenotypic characters (colour, shape, size, seedlessness),
    - purpose (table, raisin, wine, juice)

- **Propagation** is achieved by vegetative techniques so as to maintain the genetic characteristics of the cultivar.
- Sometime they demonstrate **'<u>bud sports</u>'**, which is a consequence of genetic variation of somatic cells leading to the occurrence of qualitative and quantitative phenotypic alteration in plants.
- After vegetative propagation of the bud sports, the new phenotype is generally maintained leading to a new variety. They continue to exhibit all other desirable characteristics of the parent.
- Selection of naturally occurring bud sports is beneficial for genetic improvement programs in grape.
- Common cultivars and the bud sports in cultivation are; Thomson seedless, Sonaka, Tas-e-ganesh, Sharad seedless, Manjri Naveen, Flame seedless, Red Globe, etc.
- Various wine varieties are in cultivation out of which Cabernet Sauvignon, Sauvignan blank, Chenin blank, Shiraz, etc. are common.

14.3.2. Vineyard establishment

Once planted, a vineyard can be economically productive up to 20yrs under tropical conditions. Therefore, vineyard establishment becomes an important aspect in ultimate productivity of orchard. Following factors have to be taken care of during vineyard establishment.

14.3.3. Land preparation

Generally trenches of at least 60cm deep are prepared along the rows that are to be filled with manure and soil. Distance between trenches may be 2.5M to 3.0M and preferably across the slope and secondly in north –south direction.

14.3.4. Erection of trellises:

Grape is a vine therefore it requires support of trellises. Various type of trellises are in use like pergola, mini-Y, extended-Y, telephone and niffin system. Selection of trellises is based on local situations, grape cultivar, and convenience of cultivation practices.

14.4. Selection of rootstock:

Rootstock selection depends on the local abiotic stressors i.e. soil properties, irrigation water availability, etc.

S.N.	Situation/ Problem	Rootstock
1	Water shortage	1103P, 140Ru, 110R, 420A, SO4, Dogridge, 99R.
2	Soil EC more than 2mmhos/com and water EC more than 1mmhos/cm	Ramsey, 140Ru, 99R, 110R.
3	Soil ESP more than 15 per cent and / or water SAR more than 8.	140Ru, Ramsey, 110 R, 1103P
4	Free calcium content of soil >12%.	Fercal, 140Ru, SO4, 420 A.
5	Chloride content of water > 4 meq/lt	Ramsey, Dogridge, 140Ru, Teleki 5C, 110 R, 99 R.
6	Poor vigor of the variety without any soil/ water problem.	Dogridge, St. George, SO4, 140Ru.

14.4.1. Grafting and frame work development:

About 4 to 5 months after plantation of rootstock the 6 to 8mm thickness of the rootstock at 45 cm height is achieved. In situ grafting is carried out generally after the monsoon and before cold season i.e. during in the month of September or October. Pre-grafted plants are also available now a day that can save the time period of about six months.

After grafting the framework development is achieved by regular training and pruning operations.

14.5. Annual production cycle of grape:

The originally temperate grapevines are managed differently under tropical conditions in India. I temperate areas the grapevine undergoes rest period during cold season. However, in tropical conditions the grapevine continues growing all the year round. Therefore, its growth is to be managed by double pruning method i.e. back pruning and forward pruning.

After April pruning or back pruning the bunch primordial develops through fruit bud differentiation. As there are no bunches during this period in the absence of sink from fruits, the food storage occurs in the grapevine parts. Thus, this this period is also called as the ‘Foundation Phase’ of the production cycle.

When the canes are matured with some stress and defoliation, October pruning or Forward pruning is carried out at 6 to 10 buds of the cane. The selective buds are pasted with Hydrogen Cyanamide (1.5-2.0 %) to break the dormancy and enhance sprouting of desired fruitful buds. The bunch undergoes through various stages from pre-bloom, flowering, cap fall, berry setting, berry growth, veraison and maturity and become ready for harvest after 120-140 days. Grape being a non-climacteric fruit, require to be harvested at perfect maturity i.e. at minimum TSS 18°Brix and acidity 0.6-0.8mg/100ml (TSS/ TA ratio: 20-30).

Canopy Standards during Production cycle:

Particulars	**Canopy Standards**
Spacing	8 x 5ft to10 x 6ft
Area/vine	40 to 60 sq.ft.
No. of canes/sq ft. (post back pruning)	0.67 - 0.75
No. of shoots/sq ft (post forward pruning)	0.75 - 1.0
No. of bunches/sq ft	0.75 to 1.0
No. of bunches/vine	30 - 40 to 45 - 60
Bunch wt. (g)	300 - 400
Yield/vine	12 kg to 18 kg
Yield/acre	12.0 MT

Irrigation water requirement of grapevines in black soil based on PAN evaporation reading:

Growth Stage	**Expected duration (DAP)**	**Water requirement (lit/day/ha/ mm evaporation)**
Foundation Pruning		
Shoot growth	1-40	4200
Fruit bud differentiation	41-60	1400
Cane maturity and Fruit bud development*	61-120	1400

121 days - fruit pruning*	121 -	1400
Forward Pruning		
Shoot growth	1-40	4200
Bloom to Shatter	41-55	1400
Berry growth and development	56-105	4200
Ripening to Harvest	106- harvest	4200
Rest period	20 days	-

Tentative nutritional requirement of grapevines:

Growth stage (Days after pruning)	N (Kg/ha)	P2O5 (Kg.ha)	K2O (Kg/ha)
Back pruning			
Pre-bud differentiation (1-30days)	80	-	-
Bud differentiation (31-60 days)	-	213.1	-
Post-bud differentiation (61-120 days)	-	-	80
Forward pruning			
Pre-bloom (1-40 days)	80	-	-
Bloom set and shatter (41-70 days)	-	106.6	-
Berry growth to veraison (71-105 days)	80	-	80
Varaison to harvest (106-130 days)	-	-	80
After harvest (Rest period > 20days)	26.6	35.5	26.6
Total	266.6	355.2	266.6

14.5.1. Physiological disorders

Grapevines exhibit various symptoms of nutrient deficiencies and physiological disorders that have economic importance in terms of quality and quantity of the produce. The physiological disorders observed are shot berries, hen and chicken disorder, bunch stem necrosis, primary bud necrosis, chloride toxicity, berry cracking, pink berries, water berries, mummification, sun burn, etc.

14.6. Important diseases and pests:

Downy mildew, powdery mildew, anthracnose and bacterial rust are the important diseases that affect grape production significantly. While flea beetle, thrips, jassids, spodoptera caterpillar, mites and mealy bug are economically important grape pests.

14.6.1. Plant protection and food safety

- Plant protection can be achieved in better way through disease forecasting based on growth stage and environmental conditions.

- Integrated disease and pest management can be achieved through viticulture practices, vineyard floor management, spraying and biological control agents.
- Annexure V, published by ICAR-NRCG, Pune as a part of pesticide residue monitoring, should always be referred to for selection of fungicide or pesticide as a food safety point of view.

14.7. To sum up

- Canopy management is a primary approach to mitigate biotic as well as abiotic stresses in orchards.
- Changing climatic conditions- crop cover strategy may become mandatory to protect from atmospheric stresses.
- Limited availability of water- "more crop per drop"- conventional drip irrigation v/s subsurface drip/ diffuser along with PRD, root volume and architecture.
- Quality of water- each drop of water causes accumulation of salts in root zone leading to edaphic stress and poor AWC (Available Water Capacity) - minimum use of water.
- Floor management in grape orchards is an important aspect and organic grape cultivation remains a topic of discussion all the time.

Chapter- 15

Extraction and Characterization of microbial derived plant growth regulator using HPLC

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15.1. Introduction

Microbial derived plant growth regulator are the low molecular weight intracellular and extracellular metabolites elaborated by microbial cells. Majority of the biologically active metabolites produced by fungi and bacteria can be potentially utilized as bio-fertilizers, biopesticides, phytostimulators, and plant strengtheners. The bacterial biomolecules are thought to be involved in plant growth through different mechanisms like modulation of root system architecture and enhanced shoot growth due to production of phytohormones including auxins and cytokinins. Additionally, bacteria also secrete metabolites like antibiotics and hydrogen cyanide, which inhibit the growth of pathogenic microbes and prevent the plant from attack of pathogens. IAA is a natural auxin which also acts as a signal molecule in microbes. It also has the ability to stimulate the gene expression in microbes; thus IAA plays the role of mutual signal molecule in plant–microbe interaction. Similarly GAs also act as signal molecules in the host plant; for instance, *Azospirillum and Bacillus* sp. Synthesizes GAs which activate the growth and development in plants.

The plant-associated microbes are known to contribute major role in the supplement of iron under starved circumstances however, the functioning mechanisms are yet to be explored. Involvement of siderophores in the iron supplement to the plant has been demonstrated, and understood to some extent. Another significance of siderophore production is enhancement of microbial colonization in the rhizosphere; particularly when the surface hydrophobicity of the microbial cells decreases considerably due to inadequate iron which finally inhibits the development of biofilm.

The microbial biomolecules also contribute crucial role in the developmental adaptations in plants under fluctuating environmental situations. The microbial biomolecules are also known to stimulate the nodulation process during the root-*Rhizobium* symbiosis. Metabolites (plant growth regulators) are mainly produced as by-products of the metabolic activities of microorganisms growing in a specific environment. There are various environmental factors potentially influencing the uptake and secretion of metabolites from, and into the surrounding environment (i.e., culture medium) such as temperature, pH, the concentration of nutrients, and so on. This chapter describes production and extraction of microbial metabolites, and their application potential in agriculture.

4.2. Production of bacterial metabolites

Production of microbial metabolites begins with growing the cells into suitable medium which can support the growth and metabolic activities of the inoculated organism. The metabolites production process from the microbes is highly dependent on the aim of the study as the important factors like composition of the medium, cultivation conditions, etc. are determined considering the same. The exometabolites produced by the microbes under optimal set of conditions can vary significantly even with the single altered factor. However it is needful to study the influence of varying physic-chemical factors on microbial metabolism. The same can be achieved by carefully controlling the physic-chemical environment during the cultivation period.

During the study of metabolites production by agriculturally important microbes, in addition to the above factors the soil environment also needs to be considered if the organism is intended to use as bio-inoculant. This can be achieved by using soil extract prepared using the soil of known physic-chemical characteristics. Similarly, stress responsive metabolites produced by microbes can also be studied by applying the stressor as inducer. For instance, the metabolites produced by microbes in response to the elevated saline conditions (salinity stress) can be induced by using higher levels of salt (NaCl) in the cultivation medium; or those produced in response to heat can be effectively induced using higher cultivation temperature. Similar strategies can be adapted for other physic-chemical stressor(s) as well.

Another crucial factor to be considered during the production of microbial metabolites is the growth rate of the organism. The growth rate of an organism decides the time of cultivation, and ultimately the extent of metabolites at a particular time of sampling. It is thus needful to have an idea regarding the growth characteristics of the organism under study in the medium to be used for production of metabolites.

4.3. Methods for extraction of bacterially derived plant growth regulators

Cautious sampling is central to the study of microbial metabolites as the method of sampling and subsequent processing has direct influence on the analyses. For instance the size of inoculum initially used while proceeding for the production of metabolites, composition of culture medium, cell density at the time of sampling, growth phase of the organism, presence of specific inducers and/ precursors in the growth medium, exposure of environmental stresses like pH, temperature fluctuations, salinity, presence of toxic/ inhibitory compounds, heavy metals, high/low nutrients, etc.

The process should begin with the restriction of the ongoing biochemical processes, so as to avoid the probable post-sampling changes. It is always beneficial to maintain low temperature while proceeding for the same. The microbial growth (cells) need to be removed, which can be achieved by simple methods like filtration/ centrifugation. Since most of the biomolecules secreted by the microbes are in trace quantities, it is better to concentrate the same by reducing the sample volume or by complete removal of the solvent medium. This can ensure not only the ease of handling but also efficient detection of the biomolecules that could not detected otherwise. Freeze drying can serve the purpose optimally in such case. Volatile fraction of the metabolites escapes in gas phase, while relatively larger portion of the metabolites remains in liquid phase (the cultivation medium), which can be extracted by implementing different techniques. The most predominantly preferred techniques for extraction of metabolites from liquid phase include chromatographic methods that make use of different techniques like ion exchange resins,

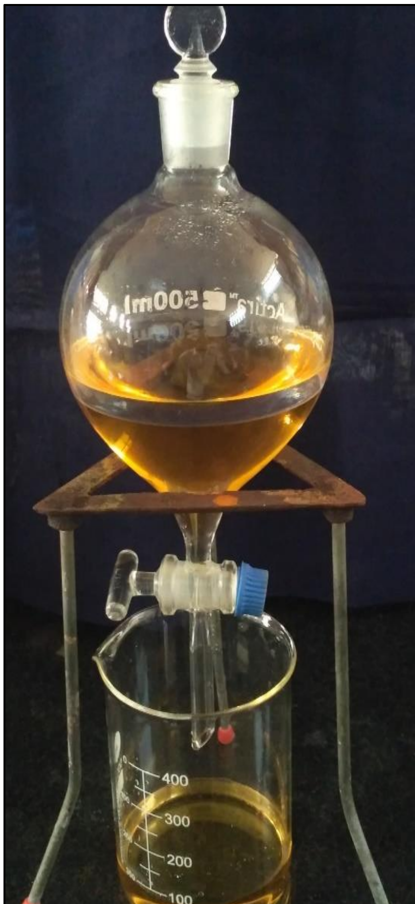

Fig. 1: Solvent based extraction of biomolecules

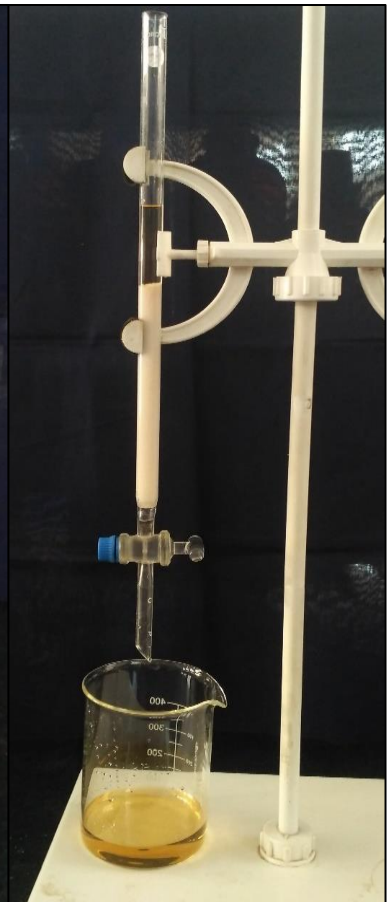

Fig.2: XAD resin based extraction of

affinity resins, solid phase extraction and solid phase microextraction, etc.; another one includes use of organic solvent like ethyl acetate, where the separation of metabolites is achieved by taking the advantage of water immiscible nature of the organic solvent; while for studying a selective class of biomolecules the use of specific organic solvents is preferred, where specific solvent is added to the dehydrated metabolites in which only selected biomolecules are soluble, whereas others are left behind in the pellet. Fig. 1, and 2 depict and overview of organic solvent, and XAD 16 mediated extraction of biomolecules respectively.

4.4. **Working protocols:**

4.4.1. Production and extraction of plant growth regulators from plant growth promoting bacteria

Plant growth promoting bacteria generally grow well in general cultivation media without requirement of specific nutritional supplements, however during the production of biomolecules, it becomes imperative to maintain optimal microenvironment for peak production of metabolites. For instance, while studying stress-responsive metabolites, it needs to maintain known intensity of the stress condition during growth of the microbes; e.g. salt concentration in growth medium, cultivation temperature, pH of the growth medium, exposure of heavy/toxic metals, etc. Similarly certain induced metabolites can also be produced by supplementing the growth medium with the specific substrate, e.g. amending the growth medium with tryptophan for higher IAA production.

The biomolecules like microbial derivatives of plant growth hormones (PGHs), different phenolic compounds, siderophores, and variety peptides and other metabolites, etc., can be studied well using UHPLC. For analytical purposes, it is needful to carry out primary separation of these biomolecules from the cultivation medium. Thus, to achieve the same, following the cultivation of PGP bacteria, the biomolecules are extracted using different methods, like extraction using resins, organic solvents (please see flow-chart below), etc. Subsequently, the extracted biomolecules are then used for bioassay, and also analyzed by different high-throughput techniques like UHPLC, LC-MS, NMR, etc. Herein we will perform the extraction of biomolecules from bacterial culture filtrates using two different methods, i.e. resin, and immiscible organic solvent – ethyl acetate.

The flow diagram explains detailed steps involved in organic solvent mediated extraction of biomolecules (Fig. 3); as well as extraction of the biomolecules using XAD resins.

pH of the solution can influence the extraction of biomolecules. It is thus needful to adjust the pH of the medium prior to proceed for extraction of specific biomolecules; e.g. microbial derivatives of plant growth hormones can be extracted efficiently at pH 2.7.

Growth of the PGP bacterium in appropriate medium under specified environmental conditions till late log to early stationary phase

↓

Pellet-out the cells by centrifugation and preserve the supernatant

↓

Extraction of Biomolecules

↓

Resin-based

↓

Mix preconditioned* resin (XAD 2, 7, 16, etc.) in the supernatant (adjust fine parameters like pH of the supernatant if specific class of biomolecules are targeted)

↓

Shake the slurry at **150 rpm / 30 min** to allow the resin to trap the biomolecules

↓

Pour the slurry in a glass column and wash with **three** bed volumes of milli Q water to remove undesired impurities

↓

Elute the trapped biomolecules with 100% acetone / methanol

↓

Remove the organic phase in vacuum at temperature ≤30 ^{0}C → Preserve at -20 ^{0}C for further studies

↓

Re-suspend the pelleted biomolecules in mobile phase and use for HPLC analysis

Organic solvents-based

↓

Use immiscible organic solvents like ethyl acetate, hexane, etc. to extract the biomolecules from supernatant. The organic solvents are mixed directly in supernatant and mixed thoroughly, followed by separation of the organic phase (in this case also, adjust fine parameters like pH of the supernatant if specific class of biomolecules are targeted)

↓

Allow the organic and aqueous phase to separate in a separating funnel

↓

Carefully separate and collect the organic phase

↓

Repeat the process and similarly perform the extraction two more times

↓

Remove the organic phase in vacuum at temperature ≤30 ^{0}C → Preserve at -20 ^{0}C for further studies

↓

Re-suspend the pelleted biomolecules in mobile phase and use for HPLC analysis

Fig. 3: Flow-diagram describing the steps involved in extraction of metabolites using organic solvent, and XAD 16 resin.

15.4.2. Preconditioning of XAD resins:

The resins may contain impurities arising out of the manufacturing and storage. The impurities may interfere with the subsequent analyses. Thus it becomes needful to make the resin free from impurities prior to use for extraction of biomolecules.

We utilize simplest method for conditioning, which makes use of milli Q water and the organic solvent to be sued as eluent. This involves washing of the resin with three bed volumes of milli Q water, then subsequent washing with the organic solvent (to be sued as eluent). The resin is then further washed several times with milli Q water to remove the traces of organic solvent. The resin is now ready for use.

The pelleted biomolecules can be utilized for further high throughput studies including NMR, XRD, mass spectrometric studies, etc.

4.5.Application of bacterially derived biomolecules in agriculture

Though majority of the underlying mechanisms involved in microbial biomolecules based plant growth promotion are yet awaiting exploration, their plant-beneficial function is gaining rapid importance nowadays. The biomolecules derived from the bacteria having plant growth promoting traits thus have importance in agriculture. It is also needful to detect the potential of these biomolecules to influence plant growth under controlled as well as *in situ* environmental circumstances. The mode of application for the biomolecules need to be optimized, particularly while applying under field conditions; e.g. application via seed coating, on leaves (foliar application), or application via irrigation, drenching, etc. Similarly dose of the biomolecules should also be optimized, following which the response of the plant at various growth stages is keenly evaluated in terms of various physico-chemical, phenotypic, molecular, yield, and quality parameters. Similarly probable spatiotemporal variations in the performance of the biomolecules should also evaluated. The results are then analyzed to formulate a *package of practice* for utilization of bacterial biomolecules in agriculture.

Economic production and extraction of agriculturally important biomolecules from plant growth promoting bacteria represents another challenging task. The production however, can be achieved with the help of keen optimization of media as well as environmental conditions. The extraction of biomolecules, as already discussed demands significant inputs both in terms of labor and cost. Particularly the solvent extraction strategy needs considerable quantity of organic solvent, increasing the overall cost several folds, which however can be controlled by recycling the solvent with the help of appropriate instrumentation and technology. Moreover scale up of production and development of efficient downstream process for large scale production will also help in achieving economic recovery of the biomolecules.

Overall, the concept of utilization of bacterial biomolecules for sustainable crop production is still in infancy. Vigorous research initiatives, particularly dedicated to *lab-to-field* approach are needed to develop new strategies and technologies for sustainable crop production under changing agro-climatic circumstances.

Chapter- 16

Ultra-High Performance Liquid Chromatography: a robust tool for analysis of microbial derivatives of plant growth hormones

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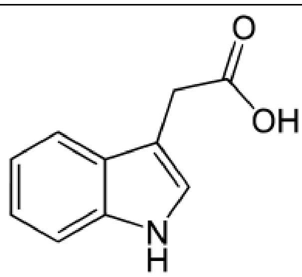
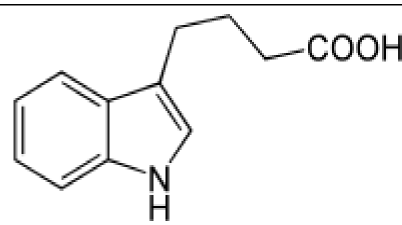
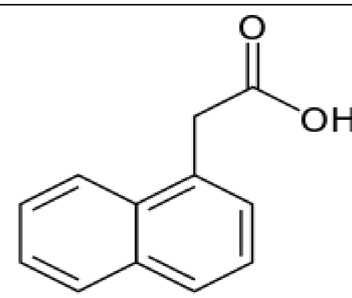
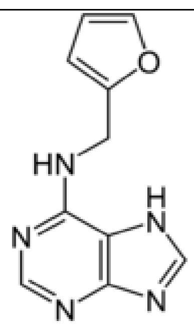
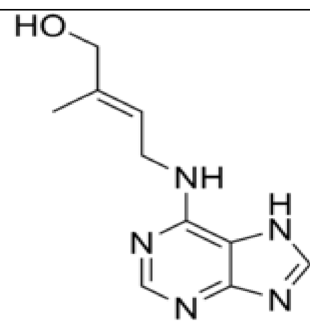
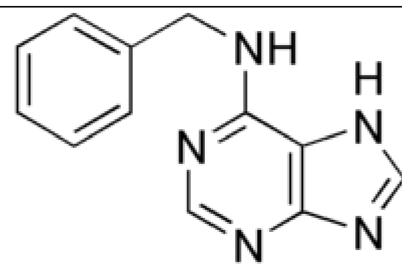
16.1. Microbial biomolecules in plant growth promotion

Microbes including bacteria, fungi and actinomycetes have been well characterized for their ability to promote plant growth through the phenomenon collectively termed as plant growth promotion (PGP). Several mechanisms are available to elucidate the microbial capabilities towards plant growth promotion, e.g. microbial production of plant growth hormones (PGHs), siderophores, volatile organic compounds (VOCs), fixation of atmospheric nitrogen, solubilization of phosphate, mineral cycling, etc. Efficient screening of the microbial strains capable of producing the biomolecules of interest in high quantities is imperative to the success of its future implementation for *in vitro* production of biomolecules or direct utilization as microbial inoculant for crop enhancement. Many biomolecules produced by plant-associated microorganisms also play important role in host-microbe signaling. Signaling molecules are important for optimal establishment and functioning of host-microbe association, as well as for maintaining cellular homeostasis in plants, particularly under abiotic stress conditions (Meena et al., 2017). High throughput estimation of these important class of PGP biomolecules is therefore critical, in light of implementing candidate microbes in sustainable agricultural practices. An array of multifaceted, cutting edge technologies are in service now a days for detection and quantitation of variety of biomolecules with high degree of sensitivity and reproducibility.

Ultra-high performance liquid chromatography (UHPLC) includes one of such equipment, capable of resolving mixtures of biomolecules with great sensitivity and reproducibility. UHPLC makes a best tool for high-throughput detection, identification, and quantification of variety of biomolecules in relatively short time. Further, the sensitivity, reproducibility and robustness of the technique can be ensured through systematic method development program. This chapter will focus mainly on different aspects related to production, extraction, and UHPLC based estimation of bacterial derivatives of PGHs under saline conditions.

16.1.1. Bacterial production of PGHs

Bacteria represent major fraction of microbial population associated with plants. The members of phyllosphere community including pink-pigmented facultative methylotrophic (PPFM) bacteria like *Methylobacterium*, and other species of bacteria such as *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Pantoea*, *Enterobacter*, etc. are known to produce PGHs in varying quantities. Major PGHs include both auxins, cytokinins gibberelins (GAs), indole acetic acid (IAA), indole butyric acid (IBA), Zeatin, abscisic acid (ABA), BAP, Kinetin (Martínez-Morales et al., 2003; Bottini et al., 2004; Kudoyarova et al., 2014; Sorty et al., 2016, Shi et al., 2017).

Auxins		
		
IAA	**IBA**	**NAA**
Cytokinins		
		
Kinetin	**Zeatin**	**BAP**

16.1.2. **Detection and estimation of bacterial derivatives of PGHs**

Traditional recipes for detection of microbial derivatives of PGHs appear principally based on the colorimetric reactions of the functional moieties harbored by PGHs. These methods make use of thin layer chromatography, colorimeter, etc. and developing reagents, e.g.

Salkovsky's reagent is extensively used for quantitative determination of IAA. However, the resolution of closely related structures remain curtained with this methods.

16.2. UHPLC mediated detection of PGHs

Liquid chromatic separation, and estimation of biomolecules have boosted the research in biomolecules several folds. Estimation of PGHs using HPLC/UHPLC has gained rapid attention owing to the high degree of accuracy and sensitivity.

16.2.1. Production and extraction of microbial derivatives of PGHs

Bacteria secrete the PGHs in surrounding environment at specific stages of growth. It is thus critical to pool the desired biomolecules from complex mixture of the cultivation medium. Further, the PGHs being metabolites, their production is highly sensitive towards the physicochemical environment. Thus ensuring controlled cultivation conditions can strengthen the probability of reproducibility. The PGHs can be pooled using specified chemical environment and organic solvents like ethyle acetate, n-hexane, resins like XAD 7, XAD 2, XAD 16, etc.

16.2.2. Pre-analysis processing of samples

The solvent content from the pooled samples should either be evaporated or appropriate blank must be used otherwise during the analysis. It is always recommended to use mobile phase composition as diluent. The samples are then diluted to avoid the excessive loading. Similarly known concentration of standard PGHs also dissolved in appropriate diluent. All the samples and standards need to be filtered using 0.2µ filter. (*The selection of filter again depends on constituents of sample. A filter should not retain any of the constituents of the sample, as it can potentially hinder with the results*). Heat labile samples should be maintained on ice bath throughout.

16.2.3. UHPLC analysis

Selection of appropriate column is needful to ensure proper results. Variety of columns are available with different specifications (*see: point 'a' in section 'e'*). Use of optimum mobile phase, flow rate and the column temperature are also crucial factors for better resolution of constituents from the samples (*see: point 'b & c' in section 'e'*). Properties of the molecules from the sample are central for the selection of detector (*see: point 'e' in section 'e'*). The samples can either be injected manually or with the help of an auto-sampler. The injection volume typically range from 0.1-50 µL for analytical UHPLC. The samples injections are done in at least 5 replicates per sample.

16.2.4. Data analysis

HPLC generates huge data following the analysis. Subtraction of blank from the mean results help in reduction of probable noise arising due to mobile phase. Peak area and height are considered for quantitative determination. The concentration of desired constituents are then calculated using standards.

16.3. Key notes

16.3.1. The column

Variety of column are available for HPLC analysis of different samples. Most crucial aspect need to be considered prior to analysis is knowledge of the sample; e.g. composition of the sample, the constituents to be resolved from the mixture of analytes, their chemical nature, structure, predicted number of constituents, expected concentration, etc. Additionally, goals of separation should also be clearly defined, e.g. whether maximum or partial resolution is needed, level of sensitivity, economy of the method, slow/fast analysis, etc. Prediction of such characters is important as it directly relates to composition of mobile phase and selection of column as well. Clearly defined goals facilitate the selection of most appropriate column that can achieve the job; e.g. shape and size of the particles in column, internal diameter, length and pore size of the column, desired surface area, carbon load, type of bonding –(monomeric/polymeric), etc can be determined more keenly. This approach can significantly reduce the time and efforts during optimization of method for a particular sample.

Most widely used column for HPLC analysis of biomolecules is **C18**. It has compatibility with diverse range of biological molecules. However, the compounds like sugar moieties can be better analyzed using **amino** columns. Therefore it is highly recommended to gain needful knowledge regarding the sample prior to proceeding for HPLC analysis. Selection of column with wide range of compatibility can suit better with complex mixtures like microbial secondary metabolites, etc.

Length of column also determines the retention time of analytes. It is thus needful to select the column of appropriate length, e.g. 50, 100, 150, 250 mm, etc. More complex analyte mixtures generally better analyzed using longer column lenght, e.g. 250 mm. Similarly, particle size of the column also is equally important; it typically ranges between 03-20 µm. Pore size, carboan load, bonding type, etc. can also be chosen similarly.

Columns have a typical pH range for optimal performance. The lower and upper pH limits of the column being used must always be considered while using mobile phases with specific pH; e.g. mobile phases containing different concentrations of formic acid, orthophospheric acid, trichloroacetic acid, etc. Exceeding the limits of pH tolerance of a column can directly affect the efficiency and life of column.

16.3.2. Mobile phase

Mobile phase constitutes an important part of successful analysis. In general, mobile phase may be a single solvent like water, methanol, hexane, etc. or it can be a defined mixture of water / buffer and miscible organic solvent like methanol, acetonitrile (ACN), etc. Composition of mobile phase e.g. ratio of organic:aqueous phase, pH, exert significant influence on retention of analytes. Thus, behavior of constituent molecules from the sample in mobile phase is another important aspect that should be addressed carefully. More specifically, the polarity of a molecule and mobile phase cumulatively determine its retention in the column. It is therefore clear that an unknown sample may demand trial and error attempts with different columns as well as mobile phases.

16.3.4. Flow rate

Retention of molecules is also significantly affected by flow rate of the samples. Flow rate is directly responsible for rise in pressure. Particle shape of the packing material in the column also has equivalent function in developing the pressure, e.g. spherical shape of the particles offer reduced back pressure when compared with that of the particles having irregular shape.

16.3.5. Column temperature

Temperature has great influence on kinetics of mobile phase, as well as behavior of the analytes. Trial and error are thus needed to discover the best separation temperature. Moreover uniform column temperature ensures a constant environment during entire analysis span. However, temperature tolerance of mobile phase, particularly of the organic component from the mobile phase, as well as of the column must be taken into account while opting high temperature range. Overall, maintaining well defined temperature environment throughout the analysis yields highly reproducible results.

16.3.6. Detection

The analytes are detected by detector. Variety of detectors are available to monitor the eluting analytes. Predominant detectors include photodiode array (PDA), refractive index (RI), ultra violet (UV), fluorescence, evaporative light scattering detector, etc. Detectors vary with respect to their working principle as well as sensitivity. It is thus recommended to acquire prior knowledge regarding the properties of analytes while selecting the detector. Selection of right detector leads to detection of the analytes with high sensitivity.

PDA is relatively more versatile and popular one, particularly due to ease of monitoring the response of analytes in both UV as well as visible range. It is more convenient with unknown analytes where it is easy to monitor the response at absorption maxima, and spectral properties of the analytes. Similarly RI detector can also be used for the purpose, however there are limitations of RI detectors particularly with respect to sensitivity, and its working only under isocratic mode.

Response of the compounds with known composition, on the other hand can be monitored using other detectors as well. Fluorescence detector represent an efficient alternative for relatively sensitive and specific detection needs. For instance, IAA can also be monitored using fluorescence detector (fluorimetric detection) at excitation wavelength of 280 nm, and emission wavelength of 350 nm (λex 280/ λem 350). This offers additional sensitivity and high degree of specificity. Similarly response of other analytes can also be monitored using literature-based data regarding their characteristic properties.

16.4. References:

1. Meena, KK, Sorty, AM, Bitla, UM, Choudhary, K, Gupta, P, Pareek, A, et al. (2017). Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies. *Front. Plant Sci*. 8:172.

2. Kudoyarova, GR, Melentiev, AI, Martynenko, EV, Timergalina, LN, Arkhipova, TN, Shendel, GV, Kuz'mina, LY, Dodd, IC, Veselov, SY (2014). Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. Plant Physiol Biochem. 83:285-91.

3. Bottini, R, Cassan, F, Piccoli, P (2004). Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. 65:497-503.

4. Martínez- Morales, LJ, Soto- Urzúa, L, Baca, BE, Sánchez- Ahédo, JA (2003), Indole-3- butyric acid (IBA) production in culture medium by wild strain Azospirillum brasilense. FEMS Microbiology Letters, 228: 167-173.

5. Shi, TQ, Peng, H, Zeng, SY, Ji, RY, Shi, K, Huang, H, Ji, XJ (2017). Microbial production of plant hormones: Opportunities and challenges. Bioengineered. 8:124-128.

6. Sorty AM, Meena, KK, Choudhary, K, Bitla, UM, Minhas, PS, Krishnani, KK (2016). Effect of Plant Growth Promoting Bacteria Associated with Halophytic Weed (Psoralea corylifolia L) on Germination and Seedling Growth of Wheat Under Saline Conditions. 180:872–882

Chapter- 17

Canopy temperature/IR thermography as a trait for phenotyping for drought and heat tolerance

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The surface temperature of the canopy is related to the amount of transpiration resulting in evaporative cooling. IR based thermometer/ camera allows canopy temperature (CT) to be directly and easily measured remotely and without interfering with the crop. It is well documented that CT is correlated with many physiological factors like plant water status, stomatal conductance, transpiration rate, crop yield etc. CT used routinely, particularly for stress diagnostic and breeding selection of stress adapted genotypes: (i) under drought conditions it is related to the capacity to extract water from deeper soil profiles and/or agronomic water use efficiency (WUE); (ii) under irrigated conditions it may indicate photosynthetic capacity, sink strength and/or vascular capacity –depending on the genetic background, environment and developmental stage; and (iii) under heat stress conditions is related to vascular capacity, cooling mechanism and heat adaptation.

CT is an integrative measurement (i.e., scoring the entire canopy of many plants within a plot), and so has advantages over other methods used for stress detection, such as stomatal conductance and water potential, because it integrates a larger area of plant/ crop measurement, is non-destructive, does not interfere with stomata (which are sensitive), and is faster and not laborious. However, trait expression shows interaction with both developmental phase and time of day (e.g., pre-heading and/or morning readings are usually lower due to lower incident solar radiation and air temperature), which can be used to relate different canopy traits and stress tolerances.

Infra-red thermometry or IR thermography measures temperature of the target by measuring the radiant thermal energy emitted by the target. Infrared is a type of electromagnetic radiation, which is emitted, to greater or lesser degree, by all objects that have temperature. IR spectral region of 8 to 13 μm is typically used for thermal remote sensing.

17.1. Factors influencing the canopy temperature

- **Stomatal features:** shape, size and structure of stomata.
- **Leaf Characters:** Number and orientation of leaves, presence of cuticle and waxiness on leaf lamina and stem

- **Plant water status:** Water content of plant/leaf in relation to that required for optimal growth.
- **Crop Yield:** high photosynthesis
- Emissivity of objects.
- **Time of day:** Measurements typically from 11:00h to 14:00h, Avoid cloudy and rainy days.
- **Environment condition:**Measurements must be in clear sky and there is little or no wind. The plant surfaces are dry and not wet from dew, irrigation or rain.
- **Plant phenological stage:** Stage should be objective based and interval should be roughly 5-7 days between each measurement– to give a reasonably heritable estimate of trait expression.

Always take measurements of the part of the plot which is most exposed to the sun, and ensure to avoid the shadow of the operator and/or shadows from the neighbouring plots.

17.2. Image capturing process in Variocam HR inspect 575

- Press the button AL. The thermographic system automatically focuses and the temperature scaling of the false-colour image is automatically optimised according to the current scene. Or Adjust the view mannualy to focus the object.
- In the live mode, joystick movements ↑, ↓ change the selected temperature level and joystick movements ←, → change the selected temperature range.
- Pressing the Enter button switches between the live mode and the focus mode.
- In the focus mode, joystick movements ↑, ↓ focus over larger or shorter distances to the object.
- For Storage of the thermal image press the S button. The live image freezes, i.e. camera goes into stop mode. Pressing the S button again saves the thermal image on the SD card. Pressung the C button interrupts the saving process. The camera will return to the live mode after the saving process.
- For switch off press button CL, the dialogue for switching off is selected and confirmed by pressing Enter.

17.3. Image processing

- From menu "File", select submenu "Open file" and open the desired thermograms (*.irb files).
- Select the desired colour palette via the "Scale", which is located on the right-hand of the thermogram.
- Via menu "View", you can display further image elements, measurement data, annotations and parameters in addition to the thermogram.
- By pressing the right mouse key on the colour scale, the dialogue "Level/Range", where the temperature level and range can be adjusted as desired by moving the scroll bar. The adjustment is also adopted for subsequent thermal images.
- With the help of the respective functional buttons on the symbol bar, points of measurement, areas, etc. as well as the display of temperature maximum and minimum can be activated.
- For inserting the analysed thermal images into the reports, select the menu "Report". Alternatively the images can be stored in common image formats suc as .jpg, .bmp etc

17.4. Prototype of tools for image based phenotyping

Taking into consideration the need to accelerate phenotyping in field efforts have been made to develop phenotyping tools. A hand operated track mounted trolley was designed for imaging purpose which hosts a camera and a Lap Top PC. The system acquires images of each plot in the experimental field after recognising the barcode. Images are stored with plot name. Tools have also been developed to rapidly analyse these images. Promising results have been obtained with image acquisition and analysis tool. This field based, semi-automated platforms potentially allow high-throughput phenotyping at a low cost.

Fig. 1. Prototypes to screen genotypes in field (a) pot culture (b) tools for rapid analysis of images (c)

17.5. IR thermometer Vs Thermal camera

- A IR thermometer gives number whereas, thermal imaging cameras generate an image.
- A IR thermometer reads the temperature of one single spot whereas, a thermal imaging camera gives you temperature readings for each pixel of the entire thermal image.
- Because of advanced optics, thermal imaging cameras can also resolve temperatures from a longer distance. This allows you to quickly inspect large areas and hance, simultaneous response recording for large set of genotypes.

17.6. Limitations of thermal imaging

- Thermal cameras are more expensive and It is greatly Influenced by being around any object and environment hence, necessitating the use of reference.
- Imaging sensor calibration and atmospheric correction are often required for high efficiency

Chapter- 18

Chlorophyll Fluorescence measurements and use in plant phenotyping

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18.1. Introduction

Chlorophyll fluorescence is one of the most highly informative, rapid and non-destructive diagnostic methods for the detection and quantification of damage in the photosynthetic apparatus caused by environmental stress. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photo-chemistry), excess energy can be dissipated as heat or it can be re-emitted as light—chlorophyll fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained. Fluorescence occurs mostly from chlorophyll a of PSII in the red region of the spectrum (685nm) and therefore it is emitted as red light. More than 90% of absorbed light is utilized by photosynthesis. Only about 1 to 2% light is utilized by the fluorescence process (Maxwell and Johnson, 2000). The spectrum of fluorescence is different to that of absorbed light, with the peak of fluorescence emission being of longer wavelength than that of absorption. Therefore, fluorescence yield can be quantified by exposing a leaf to light of defined wave-length and measuring the amount of light re-emitted at longer wavelengths. Several research documents are available in support of chlorophyll a fluorescence to assess PSII status under light (Luttge 2000), cold (Koscielniak and Biesaga-Koscielniak 1999), heat (Srivastava and Strasser 1997; Bukhov and Carpentier 2000), and water stress (Georgieva *et al*. 2005, 2007; Goltsev *et al*. 2012).

18.2. Fluorescence parameters

Chlorophyll fluorescence can be used as a non-intrusive method of monitoring photosynthetic events. There have been very many fluorescence parameters defined in the literature. The aim is to provide information on the parameters that can be usefully used in crop improvement programmes to identify differences in plant performance non-destructively and rapidly. Consequently, the focus will be on the fluorescence parameters associated with the induction of fluorescence on exposure of dark-adapted leaves to light and the operation of photosynthesis under growth and other light conditions. It gives a good measure of the photochemistry and electron transport rate and can be related to photosynthetic efficiency.

For example, environmental stresses affect the PSII efficiency and there is decrease in Fv/FM. The fluorescence parameters (as given in Table1 and references) are very useful in monitoring and judging the physiological state of the plants under environmental stresses such as water deficit, temperature, nutrient deficiency, polluting agents, attack by pathogens.

There are two types of chlorophyll fluorescence meters – time resolving (Continuous Light) fluorimeters and pulse modulated fluorimeters (Hall et. al. 1993). Time resolving fluorimeters give the fluorescence parameters (F and Fo) for dark adapted leaves and only record Kautsky curves. However, the fluorescence measurements of the light adapted leaves require pulse modulated fluorimeters. Commercially available fluorimeters as well as integrated photosynthesis systems with fluorimeter are available for monitoring the fluorescence parameters. The fluorimeters models are PEA (Hansatech), PAM-2000 (Walz) etc. The models of integrated photosynthesis systems with fluorimeter include LI-6400 (LICor), CIRAS-2 (PP-Systems), HCM-1000 (Walz) , LCpro+ (ADC).

Table1. Fluorescence parameters and their physiological relevance (Baker, 2008)

Parameter(s)/ Definition	Physiological relevance
F= Fluorescence emission from dark adapted leaf F′=Fluorescence emission from light adapted leaf	Provides little information on photosynthetic performance because these parameters are influenced by many factors. F′ is sometimes referred to as Fs′ when at steady state
Fo=Minimal fluorescence from dark adapted leaf Fo′=Minimal fluorescence from light adapted leaf	Level of fluorescence when QA is maximally oxidized (PSII centers open)
Fm=Maximal fluorescence from dark adapted leaf Fm′=Minimal fluorescence from light adapted leaf	Level of fluorescence when QA is maximally reduced (PS II centers closed)
Fv=Variable fluorescence from dark-adapted leaves Fv′=Variable fluorescence from light adapted leaves	Demonstrates the ability of PS II to perform photochemistry (QA reduction)

Fq′= Difference in fluorescence between Fm′ and F′	Photochemical quenching of fluorescence by open PS II centers.
Fv/Fm = Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by PSII is used for reduction of QA.
Fq′/Fm′=PS II operating efficiency	Estimates the efficiency at which light absorbed by PS II is used for QA reduction. At a given photosynthetically active photon flux density (PPFD) this parameter provides an estimate of the quantum yield of linear electron flux through PS II. This parameter has previously been termed *ΔF/Fm′* and *φ* PS II in the literature.
Fv′/Fm′=PS II maximum efficiency	Provides an estimate of the maximum efficiency of PS II photochemistry at a given PPFD, which is the PS II operating efficiency if all the PS II centers were ‘open’ (QA oxidized).
Fq′/Fv′=PS II efficiency factor	Relates the PS II maximum efficiency to the PS II operating efficiency. Nonlinearly related to the proportion of PSII centers that are ‘open’ (QA oxidized). Mathematically identical to the coefficient of photochemical quenching, *qP*.
NPQ=Non photochemical quenching	The non photochemical quenching from Fm to Fm′. Monitors the apparent rate constant for heat loss from PS II. Calculated from (Fm/Fm′)–1.
qE=Energy-dependent quenching	Associated with light-induced proton transport into the thylakoid lumen. Regulates the rate of excitation of PS II reaction centers.
qL=Fraction of PS II centers that are ‘open’	Estimates the fraction of ‘open’ PSII centers (with QA oxidized) on the basis of a lake model for the PSII photosynthetic apparatus. Given by (Fq′/Fv′)(Fo′/F′)

φF=Quantum yield of fluorescence	Number of fluorescent events for each photon absorbed

18.3. Setting of instrument before start of experiment

- Focus & Zoom:Place a testing plant under the FC. The testing plant should be similar to the plants that will be used in. Increase El. Shutter and Sensitivity to see a nice image. Change False- color scale (right mouse click on the scale right to the image) to Black & White. Zoom and Focus the objective to see sharp image. FOCUS and ZOOM should be kept during the whole experiment otherwise the level of absolute fluorescence signal could be changed. Then arrange the plant to final position, leaves of interest perpendicular to camera.
- Camera settings: Let measuring flashes switched on and adjust El. Shutter and Sensitivity in LIVE WINDOW. Change False- color scale to Extended spectrum or Extended spectrum 3_0_3 (the most sensitive color scales for human eye). Keep El. Shutter as low as possible (low resolution CCD between 0-1, high resolution CCD between 1-2), otherwise measuring pulses would be too strong causing actinic effect. Adjust Sensitivity by trucking the bar to get a signal in the range of 200-500 digital units (dark blue or blue color).
- Light settings – ACTINIC LIGHT: Choose intensity of Actinic light (Act1 or Act2): (a) either desired absolute light intensity can be chosen with respect to cultivation conditions, or (b) it can be adjusted according to the fluorescence transient. (a) Place a light meter under FC to the position and distance
- Protocol: Click on the magic hat pictogram in top panel – Protocol & Menu Wizard and choose measuring protocol. There are some predefined protocols on the left side. User alone can define own protocols by using Wizards on the right side. The predefined protocol Fv/Fm Page 2 is a simple protocol determining Fo, Fm and Fv/Fm. This can be used to check if Saturating pulse is strong enough before running any other more complicated protocol.
- Importing settings: Click button Use in the bottom of LIVE WINDOW to import camera and light settings to the protocol.
- Measure: Click red flash icon, Start Experiment, in the top panel to run the measurement.

Methods to achieve dark adaptation

Sr. No	Method	Limitations
1	A leaf can be put into a leaf clip shielding it from ambient light.	If the ambient light intensity is high, and the leaf is not entirely flat, there is a chance that some stray light reaches the shielded area
2	Detached leaves can be kept for a while between wet filter paper	Consequences for the physiological state of the leaf
3	Measurement in dim light under lab conditions	Leaves can still absorb and use most of the green light for photosynthesis
4	Measurements directly in the field at nigh	Measurements differ from measurements following a relatively short dark-adaptation

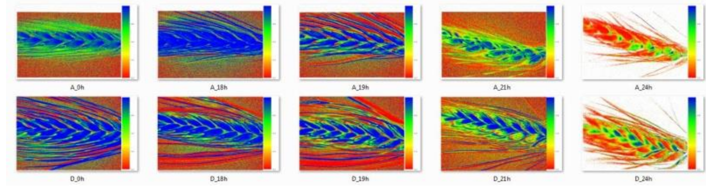
18.4. What can go wrong during a fluorescence measurement on leaves?

- Unopened or partially opened leaf clips
- Clip may shift in smooth leaves while attaching measuring head.
- Stray light may enter the leaf clip if leaf is not flat

18.5. Chlorophyll fluorescence: NIASM initiative

18.5.1. Photosynthetic system (PSII) of spikes of T. durum is more tolerant than that of *T. aestivum.*

Chlorophyll fluorescence based photosynthetic efficiency was measured in spikes of two T. aestivum and three T. durum wheat cultivars which were developed in central zone of India. It was observed that the T. durum wheat had high photosynthetic efficiency than T. aestivum as indicated by chlorophyll fluorescence parameter (Fv/Fm) at similar phenological stage. In addition, the rate of decline in photosynthetic efficiency with increase in desiccation was high in T. aestivum than in T. durum. Similar trend was observed in each of the spikelets except terminal ones. Durum wheat had relatively less moisture than the aestivum throughout the measurements suggesting that better photosynthetic efficiency in the former than in the later was intrinsic. The results also indicated that chlorophyll fluorescence of spikes could be employed for phenotyping responses of wheat germplasm for drought tolerance.

T. aestivum	
T. durum	

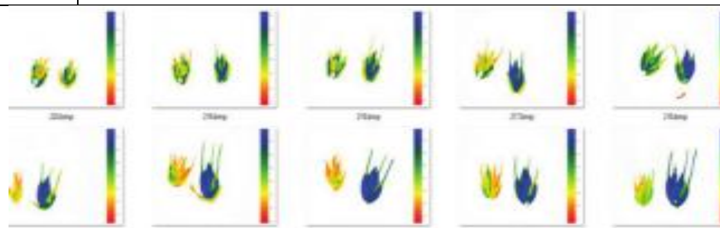
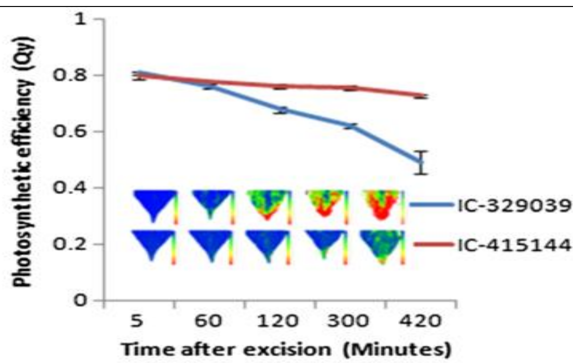


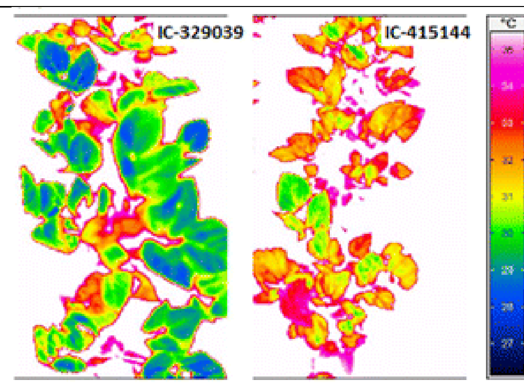
Fig. 1. Chlorophyll fluorescence imaging to assess desiccation tolerance

18.5.2. Photosynthetic system (PSII) of excised leaf of high and low LWL mungbean genotypes.

Variation in chlorophyll fluorescence in excised leaves of high and low LWL genotypes were studied. The initial fluorescence was identical (0.8) in both the high and low LWL genotypes. However, high LWL genotype recorded a sharp reduction in photosynthetic quantum yield within a period of 7 h of excision while low LWL genotype was able to maintain its fluorescence 48% higher than the high LWL genotype. The decrease in the chlorophyll fluorescence of high LWL genotype is further evident by a faster disappearance of the blue colour pixels from its leaf image while low LWL genotype reveals preponderance of blue colour pixels even up to 7 h post excisions



(a)



(b)

Fig. 2. Fluorescence images depicting change in chlorophyll fluorescence of excised mungbean leaves of high and low LWL genotypes over a period of indicated time points (a) Thermal images of the high and low LWL genotypes exposed to drought (b)

18.5.3. Photosynthetic system (PSII) sensitivity of dragon fruit to temperature was less than that of other fruit crops.

We used Chlorophyll fluorescence technique to identify fruit crops (pomegranate, sapota, sweet orange, grape, karonda, acid lime and mango) tolerant to desiccation. Photosynthetic efficiency in terms of Fv/Fm was measured in five leaves of each of the above fruit crops. Results revealed that photosystem of pomegranate were less sensitive to desiccation when compared with the same in other crops under this experiment. High sensitivity to desiccation was conspicuous in Mango as revealed by rapid decline in Fv/Fm values which indicate sensitivity of plants to stress. The rate of decrease in quantum efficiency with moisture stress was in the order of karonda < acidlime < sweetorange < grape < sapota < mango indicating that karonda was more tolerant than others.

18.5.4. Photosystem of pomegranate were less sensitive while sensitivity to desiccation was conspicuous in Mango

We employed Chlorophyll fluorescence imaging to study sensitivity of 11 different fruit crops viz; acid lime, karonda, sweet orange, grape, jamun, pomegranate, sapota, mango, guava, custard apple and dragon fruit to temperature. Chlorophyll fluorescence imaging technique was preferred for phenotyping for photosynthetic efficiency of plants based on photosystem performance. We conducted experiments with leaves of different fruit crops mentioned above clearly revealed that chlorophyll fluorescence ratio (Fv/Fm) decreases as temperature increased but with varying degree of sensitivity among the fruit crops. Studies revealed that sensitivity of photosystem of dragon fruit to temperature was less than that of other fruit crops. The rate of decrease in quantum efficiency with rise in temperature was in the order of dragon fruit< acid lime < karonda < sweet orange < grape < jamun < pomegranate < sapota < mango < guava < custard apple indicating that acid lime was more tolerant to temperature than others.

Chapter- 19

Biotechnological approaches for Climate-Smart perennial fruit crops

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The agricultural production and cultivation practices of all major crops is affected by climate change, the level of which depends on geographical areas. The climate variables such as increased mean temperatures, increase in frequency and magnitude of extreme weather events like rainfall, high temperatures, hailstorm etc. directly affect agricultural production. Perennial fruits crops are mainly selected for improved fruit yield and quality.

The adverse conditions are impacting productivity by affecting overall plant performance as well as influencing phenology of perennial crops. Influence on phenology leading to extended growth season has adverse effect not only on productivity but also on quality of the produce. Moreover, changing pattern of climate would result in increased attack of pathogens and pests and change in disease and pest epidemiology. However, not all changes in climate have adverse effect, for example increased atmospheric CO_2 and extended growing season could be favorable for improving productivity of some species. Medlyn (2011) reported 21% decrease in stomatal conductance and transpiration in woody plants in parallel to rise in CO_2 concentration, resulting in improved water use efficiency and subsequent improvement in plant performance under water stress conditions. However, a concomitant increase of ambient temperature will increase evapotranspiration and the potential gain in yield caused by the higher CO_2 levels would be compensated or result in net losses due to the negative effect of high temperature (Vara Prasad et al. 2005). It is also predicted that the elevated CO_2 levels will reduce the nutritional quality of many crops.

The severity of the effects of increased average temperature on perennial crops will depend on the phenological state. In winter, increased temperature will result early occurrence of phenological events such as flower bud induction and during the fruit development phase, higher temperatures could affect final yield and quality. Besides high temperature, other associated stresses like moisture stress, salinity, radiation etc. are predicted to affect the plants adversely and warrant immediate attention to develop technologies to manage and temperatures are predicted to have effect on precipitation.

Biotechnology has potential to mitigate effects of climate change. Genetically modified herbicide resistant crops require zero tilling thus significantly reduce fuel use, carbon sequestration and emission of CO_2 besides reducing soil erosion. Similarly insect resistant crops require fewer pesticide sprays, thus resulting in saving of fuel and reduced CO_2 emission. According to an estimate in 2011, biotech crops resulted in reduction of 37 million kg of active ingredients, decreased rate of herbicide and insecticide sprays and ploughing reduced CO_2 emission by 23.1 billion kg of CO_2 (source: https://www.isaaa.org/resources/publications/pocketk/43/default.asp). Attempts are also being made to develop plants that can store more carbon dioxide in their roots. Another application of biotechnology is the development of varieties with resistance to biotic and abiotic stresses either through transgenic or marker assisted breeding. While success has been achieved for annual crop, little or no head way is achieved in perennial fruit crop.

Perennial life cycle, genetic heterozygosity, self-incompatibility, large body size and lack of knowledge of genetics of most traits are major constraints faced by the researchers involved in genetic improvement of fruit crops. Moreover, understanding of fruit development physiology and metabolic pathways as well as identification of key genes for important traits are prerequisite for developing climate resilient varieties. In fruit crops, so far, only the functions of a few genes are known, thus hampering the progress to large extent. Understanding of plant response to stress is important to develop strategies for cultivar improvement as well as for stress management. Big strides have been made in the development of techniques and instrumentations for big data generation and analysis of genomic, transcriptomic, proteomic and metabolomics data. The collective term used for the data obtained by global analysis of genome, RNA, protein, metabolites etc., is Omics. Integration of omics data with phenotype and genotype data enables identification of genes involved in different traits and also allows understanding of effect of different environmental factors on biological process.

Genomics plays an important role to improve the efficiency of plant breeding for sustained production under changing environmental conditions by allowing efficient exploitation of adaptation traits present in germplasm accessions and by expanding the gene pool. Availability of genome sequence facilitate the identification of candidate genes for tolerance. On the other hand, Next generation sequencing techniques allows identification of SNPs which could be used for identification of genomic regions for traits either through QTL mapping or marker trait analysis and subsequently closely linked markers for traits related to tolerance to different stresses. In non-model crops where genome sequence is not yet available, transcriptome analysis is considered to be a viable approach to understand the gene function and molecular basis of many cellular responses of plants in response to abiotic stress. For non-model plants, *De novo* assembly of short RNA sequences is used to reconstruct the entire transcriptome and gene expression profile at the temporal and spatial basis to identify the key genes. Proteomics approach is frequently used to identify global changes in structure and abundance of proteins in response to environmental signals. Proteomics has the advantage of its capacity to reveal post-translational modifications, which is important to estimate the impact of modification on plant productivity. Systems biology approaches to integrate genomics studies with omics data related to a particular phenotype pave the way to interpret the complex quantitative traits.

As a case study, identification of genes for salt, moisture and heat stresses, which are important manifestations of climate change, in grapes using omics approaches will be discussed. Understanding mechanism of plant response to salinity is important to develop strategies not only for cultivar improvement but also for stress management. In different studies, whole transcriptome analysis of grapevine leaves in response to salt, heat and moisture stresses identified a large number of differentially expressed genes (DEGs). The placement of these DEGs on grape chromosomes indicated clustering of genes on chromosomes suggested putative QTLs for salt stress tolerance. Besides, pathways such as metabolic pathways, biosynthesis of secondary metabolites, membrane transport development related pathways were the major pathways affected by salinity stress. Microsatellite markers were also identified in selected DEGs and will have direct application in the development of varieties for improved tolerance.

19.1. References

Medlyn BE (2011) Comment on “Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 Through 2009”. Science 333:1093-1093. 10.1126/science.1199544

Vara Prasad PV, Allen LH, Boote KJ (2005) Crop Responses to Elevated Carbon Dioxide and Interaction with Temperature. Journal of Crop Improvement 13:113-155. 10.1300/J411v13n01_07

Chapter- 20

Mini-rhizotron technique for in-situ root study

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Monitoring of root architectural developments holds potential scope for the exploitation and desirable manipulation of root characteristics to enhance resource-use efficiency and crop productivity. Much emphasis has been to quantify the behavior of above ground development of the plants, and below ground developments of roots have been neglected in most of the field experiments conducted so far. This discrepancy might be ascribed to their hidden nature at below ground and of variable nature, both of which enormously complicate observation and conducting the research trials and not easily instrumented or observed due to relatively high cost and technical difficulties in sampling, data collection and analysis. Though, advance techniques and tools are required to record the complex and vital process of root architectural development for a desired period or at a particular critical crop growth stage. The growth and behavior of roots can be monitored for an entire crop growing season through successive measurements on the same site and same depth by mini-rhizotron technique. The in-situ root images are analyzed with an available root analysis software package and data thus collected from the mini-rhizotrons can be used to draw a dynamic picture of root system in terms of root count, length, diameter, surface area and volume. It is a non-destructive but expensive method for root studies.

20.1. Equipment and in-situ root monitoring

20.1.1. Image acquisition software

The system (CI-600 and CI-601 Root scanner) for automating image capture for mini-rhizotrons has recently been developed by CID, Inc, WA, USA (Fig. 1). With the introduction of the CI-601, image acquisition can be performed automatically and remotely.

20.1.2. Mini-Rhizotrons

The clear acrylic tubes having 6.4 cm inner diameter and 7.0 cm outer diameter and 105 cm standard length are used for installation in the field (Fig. 1). However, tubes with greater length (180 cm) are also can be utilized.

Fig. 1. Mini-Rhizotron for in-situ root image acquisition

20.1.3. In-situ root image acquisition using the CI-600:

The CI-600 root scanner connected with a laptop computer through USB as shown in Fig. 2 is inserted into already installed rhizo-tubes to monitor root growth at a desired depth. When the plant begins to build a network of roots, images of the structure and behavior of the roots can be recorded. The CI-600 scanner head rotates within the tube to scan roots and it provides nearly 360^0 high-resolution images of soil and roots of 21.59 x 19.56 cm size. The connected images of different depths can be captured by moving the camera along the tube and these images can be saved in computer for further analysis.

Fig. 2. In-situ root image acquisition by using CI-600 root scanner.

20.2. Image analysis system

Image analyses systems provide an opportunity to facilitate analyzing procedure. They offer a rapid assessment of root characteristics like length and surface area, diameter and tips, root branching patterns etc. The image analysis software "CI-690 RootSnap!" has been developed by CID, Inc, WA, USA. It allows users to measure root growth and turnover dynamics, disease, and behavior over time by analyzing scanned images collected with the CI-600. The analysed images are stored in rsp formats and the software supports exporting data to Microsoft Excel for further statistical analysis.

RootSnap! is a faster and more reliable method for analyzing root images. It includes a revolutionary user interface that employs a combination of advanced image analysis and a multi-touch LCD screen, which allows users to quickly and easily trace roots using their fingers.

20.2.1. Analysis of in-situ root images with CI-690 RootSnap! Software:

After the RootSnap! software is installed on a touchscreen computer, the application can be accessed from the Start Menu or by double-clicking on the RootSnap! icon on the desktop.
To begin using RootSnap!, an image of a root needs to be imported into the program or an already saved project or session can be opened. The Menu Bar displays File, Edit, View, and Help and many other features.

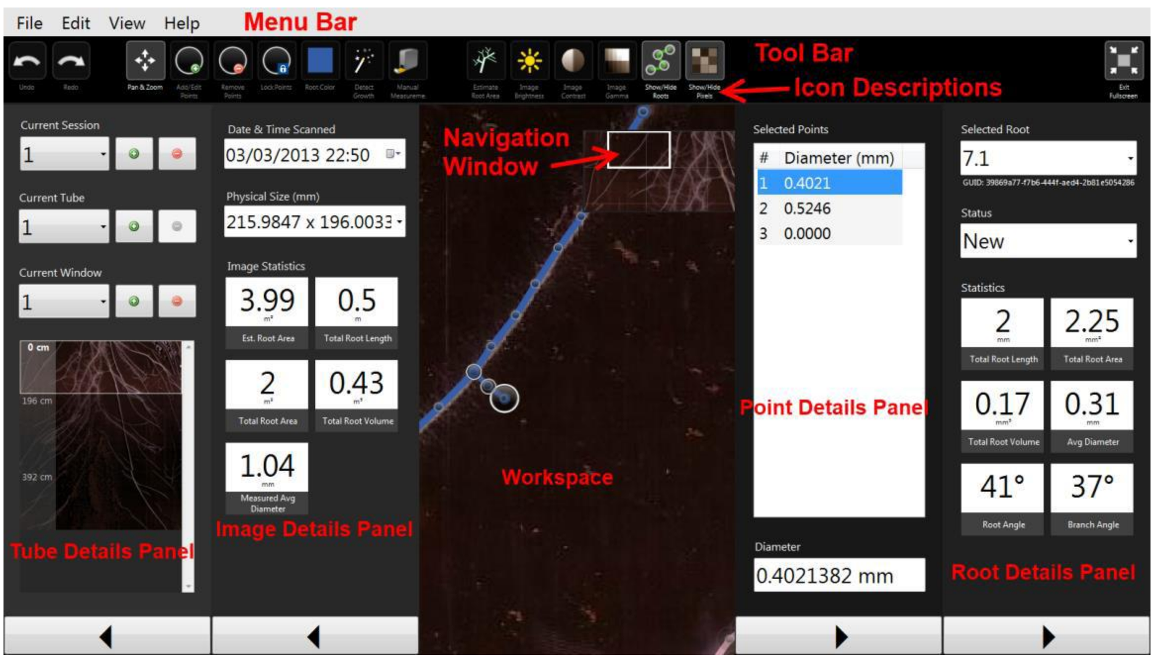
Edit Menu:	**View Menu:**	**Help Menu:**
1. Undo & Redo 2. Delete current root 3. Lock current root 4. Migrate roots 5. Tube angle 6. Default root status	1. Navigation window 2. Zoom 3. Panels 4. Layers 5. Toolbar 6. Icon descriptions 7. Large icons	

20.2.2. Tool bar options:

- Undo & Redo
- Pan and Zoom

- Add/ Edit Points
- Range
- Snap to Root:

  When this feature enabled, a root drawing that has just been traced will automatically "snap" to the center of the actual root. The center of the root is determined by the matching the identical color as the last root point, within the range of the tool, for the next root point.
- Auto Detect: The Auto Detect tool will automatically find roots using the Snap To Root application of RootSnap!



20.2.3. Beginning to Map Roots:

Select a window image to start mapping. Follow the steps as given below for mapping the roots:

1. Import image.
2. Rotate/flip the image.
3. Verify correct physical size in Image Details Panel.
4. Window alignment (if lower in tube then Window 1).
5. Adjust image Brightness, Contrast and Gamma
6. Estimate root percent.
7. Zoom in on root to map until it is at least as wide as your finger.

8. Place the first point in the center of the root. The first point is critical; it must be on the root!
9. Detect growth.
10. Check automatically mapped points for accuracy and diameter.
11. Move/place points past color change to keep automatically detecting growth.
12. Detect growth again.
13. Start mapping branches:
    a. Map a few points and detect growth.
    b. Dock branch to parent root.
14. Detect growth.
    a. Move points to end of branches.
15. Continue for rest of root system.

20.2.4. Exporting Data

Data from RootSnap! projects, tubes, windows or sessions can be exported to be opened as a spreadsheet. Exported data is saved as .csv (comma separated value) files which can be opened using Microsoft Excel or similar programs and saved. Root data is displayed at the top of the file including the root id, length, average diameter, area, volume, mean angle, branch count, branch ids and point count. Option is also available to save the root image while exporting the data.

20.3. References

CI-600 manual, CID, Inc, WA, USA , https://cid-inc.com/plant-science-tools/root-measurement-with-minirhizotron/ci-600-in-situ-root-imager/.

CI-690 Rootsanp manual, https://www.cid-inc.com/plant-science-tools/root-measurement-with-minirhizotron/ci-600-in-situ-root-imager/ .